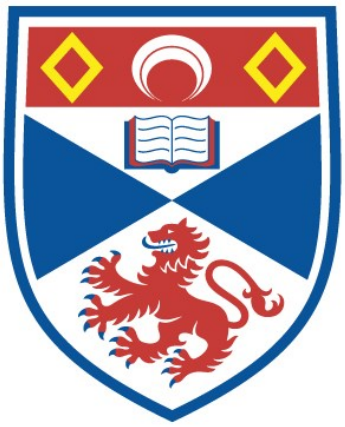
SALT REGULATION IN ARMERIA MARITIMA (MILL.) WILLD

Andrew W. Hawkins

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1970

Salt Regulation in *Aryanis maritima* (Mill.) Willd.

by

Andrew W. Hawkins, B.Sc.

A thesis submitted to the University of St. Andrews for the degree of Doctor of Philosophy.

Department of Botany,
St. Salvator's College
University of St. Andrews.

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Declaration

I hereby declare that the following thesis is based upon work done by me, that the Thesis is my own composition, and that it has not been previously presented for a Higher Degree.

The research was carried out at the Gatty Marine laboratory, University of St. Andrews, under the supervision of Dr. D.C. Weeks.

Career

I first matriculated at the University of St. Andrews in October, 1962, and in June, 1966 was awarded a Class II, Division I, Honours Degree in Botany. I was awarded a Science Research Council Postgraduate Research Studentship for 3 years research at St. Andrews.

In October, 1966 I was admitted as a research student under Ordinance General No. 12, and later as a candidate for the degree of Ph.D. under Resolution of the University Court 1967. No. 1.

Certificate

I certify that Andrew William Hawkins has spent thirteen terms of research under my direction, that he has fulfilled the conditions of Ordinance General No. 12 and Resolution of the University Court 1967, No. 1, and that he is qualified to submit the accompanying Thesis in application for the Degree of Doctor of Philosophy.

Acknowledgements

The author wishes to record his gratitude to Dr. D.C. Weeks of the Department of Botany, University of St. Andrews, for supervising the work presented in this Thesis, and for his encouragement and enthusiasm throughout the project.

He also wishes to thank Professors J.A.Macdonald of the Department of Botany, and M.S.Laverack of the Department of Marine Biology, for providing facilities for research; the technical staff of the Gatty Marine Laboratory, especially Mr. J. Brown, for their help and assistance; and Dr. P. Boden and Miss. S.A. West of the Department of Geology for their analysis of a serpentine rock sample.

He is also pleased to acknowledge the Science Research Council for the award of a post-graduate Studentship, the Master of St. Salvators College for a maintenance grant in the later stages of research, and the management of Rowntree-Mackintosh & Co. Ltd., for providing time in which to conclude the writing.

Dedication

The work presented in this thesis is dedicated to my parents and friends, without whose support and encouragement the report might never have been completed.

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Abstract

Estuarine and montane plants of Armeria maritima (Mill.) Willd., differ slightly in anatomy. In their natural environment estuarine plants are frequently subjected to substantial variation in the total salinity of the root medium, while montane plants are not. Salt regulation in the two races was studied comparatively, using an electrochemical approach.

The plants were grown in the laboratory in dilute culture, and subsequently exposed to increasing salinity up to the concentration of artificial sea water, the relative cationic composition of which was varied. The electrical potentials between the leaf water free space (W.F.S.) and the bathing media were measured, as were concentrations of the major ions in the W.F.S. and the bathing media, and the data applied to the Nernst and Ussing-Teorell equations. Comparison of predicted with actual fluxes obtained using radio-tracers showed active export of Na^+ and uptake of Cl^-. K^+ appeared to be actively imported at low external concentrations and passively distributed, with the possibility of active export at high and very high ambient concentrations.

On the basis of purely electrical studies, a hypothesis is proposed for the role of the root in controlling the internal ionic environment of these plants with changes in the concentration of the bathing medium.

The mode of action and efficiency as de-salinators of the salt glands present in the leaf are considered.

Introduction

Chapter 1.

Armeria maritima (Mill.) Willd., commonly known as sea pink, or thrift, is a member of the Plumbaginaceae occurring as a frequent inhabitant of coastal salt marshes and occasionally on isolated rocky outcrops in the mountains. (Clapham, Tutin and Warburg, 1951). There appears to be no intermediate distribution unless the plant is grown in artificial habitats where competition is minimised, e.g. in rock gardens.

In its natural environment, the plant appears to be capable of withstanding extreme conditions. The more usual habitat, the salt marsh, is periodically submerged in salt water, which may be followed by periods of prolonged sunlight, causing considerable evaporation of soil water and hence concentration of the surrounding saline, or prolonged heavy rain, causing the soil solution to become diluted. Thus plants growing in this habitat must be able to withstand high ionic concentrations, and the osmotic stress caused by increase or decrease in the osmotic potential of the ambient medium. In contrast, the montane plants described in this study live in open communities on alkaline serpentine rock, containing a high (35–40) percentage of magnesium. The roots of the plant are, however bathed in an acidic (pH 4.6) soil solution, which

has presumably been leached from a large peat bog higher up the mountain. The proportions of sodium and potassium in the rock are small but not insignificant.

Thus the plants occupy very different habitats but are morphologically almost identical. Godwin (1956) has described the calyx tubes of the montane and estuarine plants as differing in the length and position of their hairs. Examination during the course of this work indicated a further small difference, in that the montane plant has double the number of stomata on the abaxial surface than on the adaxial surface of the leaf. Numbers of stomata on the adaxial surfaces of plants from the two habitats were similar, as was the distribution of the typical plumbaginaceous glands.

Plants growing in an environment which is subject to sudden changes in its osmotic potential, as is the case with Armeria maritima growing in salt marshes, must be capable of considerable control over their internal salt status, such that the normal metabolic functions of the plant are 'buffered' against alternate dehydration and dilution. The aim of this work was to ascertain whether this regulation was brought about by active exclusion of all passively entering ions, at the risk of turgor loss; by active absorption of ions, followed possibly by re-excretion when an ion 'pool' of sufficient magnitude to prevent dehydration

had accumulated; by control of the ion entry by change in membrane permeability to each major ion with change in external concentration; or by a combination of these mechanisms. Montane plants were included in the study in order to ascertain whether, because of their similar morphology, they possessed similar mechanisms for ion regulation.

Many studies on ion regulation have been inconclusive because they have been made on the assumption that active accumulation or exclusion can be defined purely as a function of movement against a concentration gradient (Lundegårdh, 1955, Hylmö, 1955, 1958, Fried and Shapiro, 1961). However, an electrical gradient also exists between a plant compartment and another plant compartment, or the bathing solution. Thus an anion can move against a concentration gradient, but along an electrical one, to neutralise a positive charge on a cation which is being accumulated. Passive equilibrium will be reached when a balance is attained between the outwardly directed concentration gradient and the inwardly directed electrical gradient. For this reason metabolic inhibitors, when used, may result in cessation of transport of both ions, with the conclusion that movement of each ion was mediated by an active process. In actual fact, however, only one ion was being constrained.

Thus it is essential that the combined electric and chemical forces operative on each major ion be elucidated, before conclusions can be reached as to the occurrence, and direction

of constraint on an ion.

Each plant is a complex of interdependent systems as each tissue grows, senesces, and regulates its own internal ionic environment. The methods to be used for determination of mechanisms of salt regulation had, therefore, to be relatively simple in application. For example an approach based on irreversible thermodynamics would be too complex mathematically and conceptually for an initial study. The immediate objective was an understanding of ion regulation, independently of water regulation, before complex interactions could be considered. For these reasons a simple electro-chemical approach was adopted, under conditions of low water flux through the plants. The general thermodynamic state under these conditions was reduced and simplified so that Nernst and Ussing-Teorell equations (Dainty, 1962) could be applied.

If passive equilibrium has been reached for each ion between an internal compartment in the plant and the external solution, the concentration and electrical gradients will be equal and opposite. The Nernst equation (below) is obeyed for each ion at flux equilibrium, which is not under metabolic constraint.

$$E = \frac{RT}{ZF} \log_e \frac{Co}{Ci}$$ where,

E = electrical potential in millivolts
Z = algebraic valency of the ion
F = 96.3 coulombs/equivalent
T = temperature, $^{\circ}K$.
R = 8.31 joules/degree/equivalent
Co,Ci = the external and internal ionic concentrations

The values of concentration referred to above are

approximations for the activities of the ions concerned in the compartments under study. The validity of this assumption and consequences of any errors incurred because of it, are discussed in the text.

If the value of E calculated from the above equation corresponds with that measured between the compartments under study, and flux equilibrium for the ion has been reached, it may be concluded that the ion is in passive flux equilibrium. Deviation from the calculated value is indicative of active constraint on the ion, the magnitude and sign of the deviation being descriptive of these active forces.

It is unlikely that flux equilibrium will ever be obtained in a complex living organism, owing to independent growth and senescence of tissues in the organism. Therefore, data must be adapted to provide a value for the expected flux ratio between the compartments if the ion being studied was being passively distributed. The Ussing-Teorell equation predicts this ratio:

$$\frac{Jin}{Jout} = \frac{Co}{Ci \exp. \left(\frac{ZFE}{RT}\right)},$$

where Jin, Jout are the partial, or one-way fluxes between the compartments. Values obtained from the equation may be compared with those obtained using radio-tracers, deviation again being indicative of constraint on that ion.

For a detailed picture of ion regulation in each tissue, the parameters of E, Co, Ci, must be measured between the

cell contents and the immediate bathing fluid. Before the salt regulation of the plant can be fully understood, such information must be obtained. However, as an initial approach it was felt that a more general knowledge, which could be used as a basis for further study, would be obtained by measuring the electrochemical gradient for the major ions between the xylem sap and the medium bathing the roots, (Bowling and Spanswick, 1964, Shone, 1968).

Values obtained from this study would not themselves be indicative of possible regulation by the exodermis, cortex or endodermis, but would be representative of the combined effect of these systems in series, if a barrier to free diffusion into the root exists at the exodermis, or of the effect of the endodermis as a final permeability barrier, if free diffusion of the medium occurs throughout the cortex.

The delicate structure of the roots and the narrow cortex, precluded separation of the stele and cortex so that uptake by these tissues could not be measured separately. It was hoped, however, that uptake of tracers by the root might yield kinetically distinct phases attributable separately to cortical and stelar uptake. The concentration of the ions in the cortex could then be estimated knowing the loss of radioactivity of the ambient medium during uptake into the cortex, and its specific activity.

The total volume of the cortex could be obtained approximately from this section, and so flux ratios could be calculated using the Ussing-Teorell equations incorporating these concentrations and electrical potentials made under similar conditions. Values of predicted and actual fluxes could then be compared. Hence it was hoped to obtain a comparison of ion regulation between the cortex and the bathing medium, and the stele and the bathing medium.

Unfortunately time did not allow for measurements of rates of tracer appearance in the xylem sap after external application.

The small size of the cells, and the structure of the root, are such that independent analysis of cytoplasmic and vacuolar concentrations is impossible by direct sampling, or by the indirect method of elution. Thus fine analytical work along the lines of the research on giant Nitella coenocytes which has led to MacRobbies' (1970) theories of vesicular transport of ions to the vacuole was impossible.

The approach envisaged was therefore, one of a general assessment of ion regulation within the plant, leading to an evaluation of the parts played by the root and leaf (glands), in the control of the internal ionic environment of the plants, with changes in the concentration and composition of the ambient medium.

For simplicity, the ions Na^+, K^+ and Cl^- will be referred to throughout as Na, K and Cl.

Plate 1

Materials and Methods

Chapter 2. Section 1.

The Plants and their culture

Plants were collected in October 1966 from the salt marshes in the Eden estuary east of Guard Bridge (Plate 1A), and from the serpentine outcrops of Meikle Kilrannoch, 2850′, in the Cairngorms north of Kirriemuir (Plate 1B). The diversity of habitat is obvious, the closely knit salt marsh community being continually subjected to submergence at high tide. The serpentine community on the other hand is very open, the roots of the plants penetrating the basic serpentine rock, but being irrigated continuously by an acidic solution (pH 4.6), draining from a peat bog a few feet higher, on the flat summit. The composition of serpentine rock has been found to be high in magnesium and silicon with a low but constant level of potassium (Green, 1964), Challis, 1965). The analysis conducted by Green on the Lizard serpentine, and Challis on serpentine in New Zealand, reveal a quite substantial variation in the amount of sodium present in the rock; 0.34% in Cornwall, and 0.06% in New Zealand.

Analysis of a rock sample from the Cairngorm serpentine carried out in the Department of Geology of the University of St. Andrews by Dr. F. Bowden and Miss S. West, revealed

0.01% Na_2O and 0.16% K_2O. Thus the sodium content of the rock is lower, but the potassium higher than the values of 0.08% found for other serpentine outcrops.

It was assumed that the roots of estuarine Armeria maritima are in continuous contact with a solution of the basic composition of sea water but whose concentration may vary (Chapter 1). Analysis of surface water from Meikle Kilrannoch revealed a solution much richer in Na than K (table 1), whose concentration varied from dilute, when surface snows were melting (a), to more concentrated at the end of a dry summer, (b).

Table 1

Estuarine				Montane		
	Na	K	Ratio (Na:K)	Na	K	Rat (Na:
Rock	-	-	-	0.01%	0.16%	1:1
Bathing solution	475mEq/l	11mEq/l	1:0.023	a)0.36mEq/l b)0.8 "	0.12mEq/l 0.28 "	1:0 1:0

The relatively high proportion of Na in the surface water bathing the montane plants, can only be assumed to have been washed out of the peat, composed mainly of decaying Carex spp. The ratios of Na:K in the respective irrigant solutions show a considerably higher proportion of K available to the montane plants.

Whole plants freshly removed from the two habitats were washed as quickly as possible to remove adhering mud, divided into

stem, root and leaf portions, ashed and analysed for the major cations using the method described in Piper (1950). The results, together with water content expressed as a percentage of fresh weight, are tabulated below (Table 2).

Table 2.

	Water(% F.W.)		Na(mEq/gmF.W)		K(mEq/gmF.W.)		Ratio Na:K	
	Es.	M.	Es.	M.	Es.	M.	Es.	M.
Leaf	78.98	80.30	14.6	5.4	8.0	15.4	1:0.554	1:2.84
Stem	70.44	78.23	11.2	2.0	8.2	7	1:0.73	1:3.53
Root	71.61	76.85	7.4	2.8	8.6	8.7	1:1.16	1:3.06

Thus montane plants contain more water than their estuarine counterparts, presumably a reflection on the lower osmotic stress afforded by their environment. As would be expected the relatively higher proportion of potassium in the environment of the montane plants is reflected in the ash analysis, although it would seem that both populations are able to select in favour of potassium. The interesting figures are those showing the similarity between the potassium content of the root and shoot of the two populations. It would seem possible that montane plants can accumulate potassium, and estuarine plants restrict its entrance to a constant level. The greater concentration of potassium in montane leaves will be discussed later, as well as the rest of these analysis in conjunction

the experimental findings.

A feature of Armeria maritima is its very low shoot to root ratio, and so great care had to be taken not to damage the rooting system whilst removing the plants from the ground. Each cluster of plants seen at the surface (Plate 1B) is often served by the same root system, there being extensive anastomosis of the roots just below ground level. Plants were selected as far as possible where there had been little anastomosis, but in some cases it was necessary to split the clone. Each individual shoot will be referred to as a 'Head' later in the account.

The root systems were washed gently in tap and distilled water to remove all traces of soil, and the plants were then supported by a fibre glass sheet and a polythene mesh over a plastic dish, in such a way that the root systems were immersed in aerated mineral culture solution of composition:-

1 mM	$Ca(NO_3)_2$
1 mM	KNO_3
0.4mM	$MgSO_4$
0.2mM	KH_2PO_4
0.2 mM	KCl

with trace quantities of H_3BO_3, $CuCl_2$, $MnCl_2$, $ZaCl_2$ $(NH_4)_2$ MoO_4, and 5 mg.Fe/ml. as iron ethylenediomine tetracetate.

The plants were left in this condition in a ventilated

Plate 2

chamber under fluorescent lighting for several months. Unfortunately they succumbed to infection by Fusarium sp. over Christmas 1966.

More plants were gathered in the spring of 1967, and were grown in aerated culture in separate blackened bottles. The plants were placed on a window bench in the laboratory (Plate 2) which unfortunately faces north, so extra illumination had to be provided. 2 Osram 65/80w warm white fluorescent tubes fitted with back reflectors were suspended 30" above the plants, and the 'day' length was controlled by a Venner time switch, to provide a minimum of 10, and a maximum of 18 hours light according to the season. The light energy received by the plants was 1.69×10^{-2} cals/min/cm^2/mμ.

The aeration was supplied through glass pipette bubblers connected through linear mannifold devices attached to the back of the bench, to 2 'Hi-flo' electrically operated air pumps. Each bubbler reached to the bottom of the culture solution, and the rate of bubbling was adjusted by means of screw clamps to between 2 and 5/second.

Each bottle had been coated previously with 2 applications of black enamel paint to prevent algal contamination of the culture solution, and the inside coated with 'Repelcote' solicone mixture to prevent 'wetting' of the glass, and hence the possibility

of contamination of the culture solution, by elements dissolving slowly from the soda glass.

The culture solution was replenished with distilled water twice a week and changed completely every 4 weeks.

It was found that by growing each plant in isolation, spread of fungal infection could be checked by removing any diseased plant. The plants were grown in culture for 6 months before being used experimentally.

Section 2

Anatomy of Armeria maritima

Some anatomical knowledge of the plant was essential in order that experimental data on function could be interpreted in the light of structural features. Metcalfe and Chalk (1950) give only passing reference to Armeria maritima in their small section on the Plumbaginaceae, so it was decided to spend a short time in sectioning the leaves and root.

Fitting (1911), and Montfort (1922) had observed crystals on the leaves of Armeria maritima, and had postulated the existence of glands on the leaf surface. Metcalfe and Chalk draw attention to the typically Plumbaginaceous glands, but their illustrations are not clear, both as to the structure of the glands, and as to their possible association with vascular tissue in the leaf.

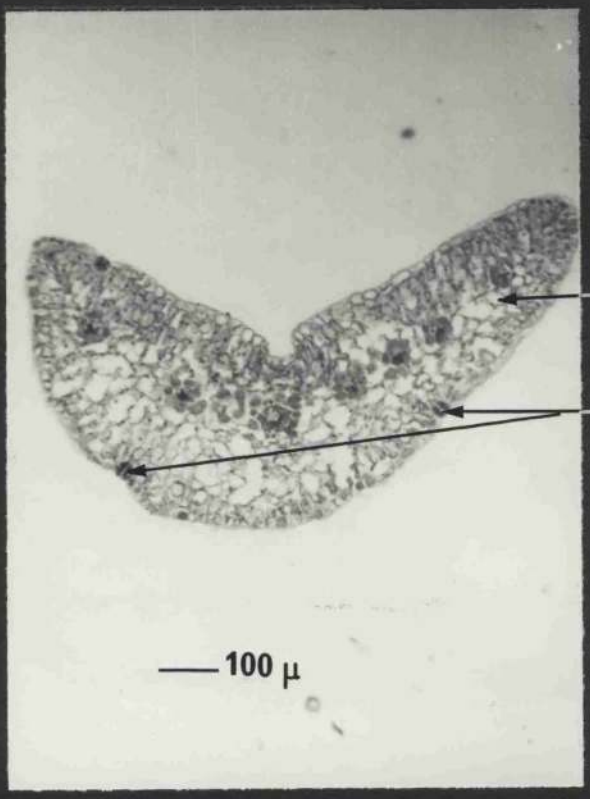
The root is obviously of primary importance as it is the organ through which solute absorption occurs. Metcalfe and Chalk mention the possession of a heavily suberised endodermis, but go no further in describing possible anatomical barriers to ion uptake.

Leaves

Leaves of both montane and estuarine specimens, which are typically grass-like in appearance being about 2-3 mms wide and 3-5 cms long, were killed in formaline-aceto-alcohol, and fixed

Plate 3

A



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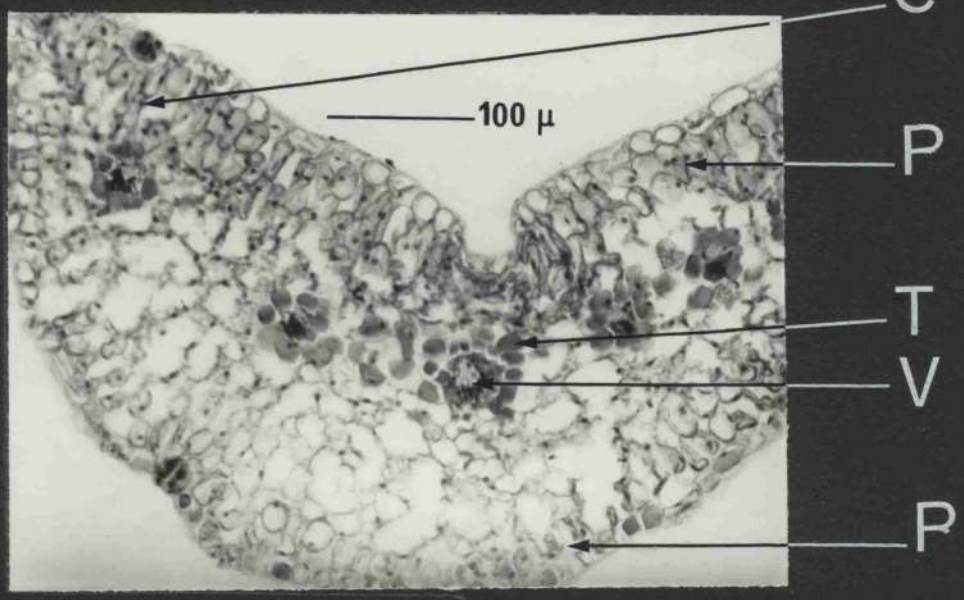


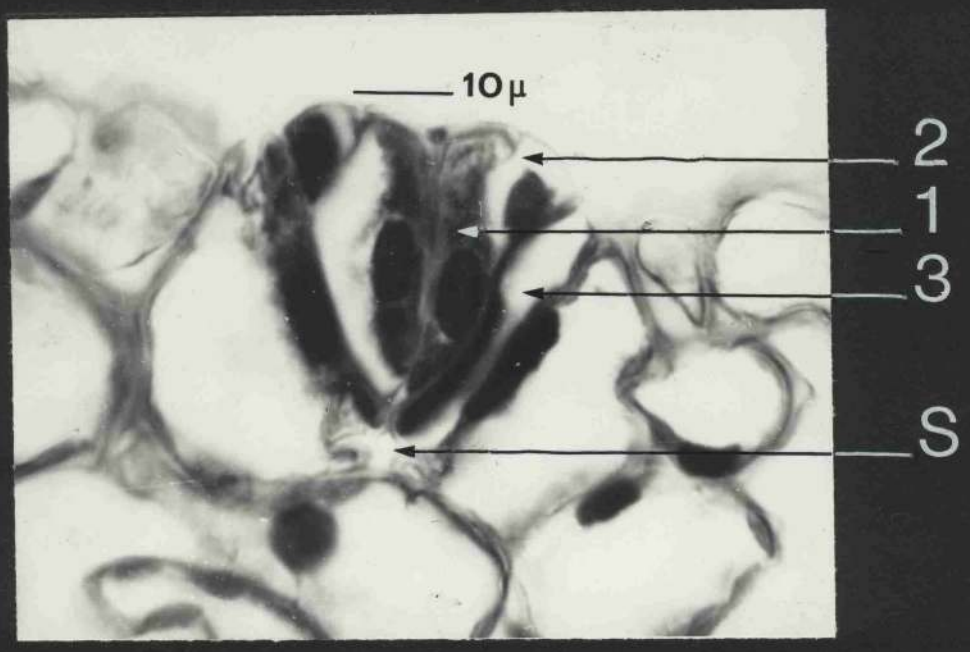
in tertiary butyl alcohol (Johansen 1940) before being embedded in paraffin wax. 5 µM. sections were cut on a Leitz Wetzlar hand operated microtome, and mounted on slides using egg albumen as an adhesive. The sections were stained in safranin and fast green (Johansen 1940), and photographed using Ilford 'Micro-neg' and 'Pan-F' films, in a camera attached to a Watson Service 3 microscope.

A typical section is shown in Plate 3 A & B.

The glands(s) occur both on upper and lower epidermal surfaces, there being no significant difference in the populations studied between the number of upper or lower surface, or indeed between estuarine and montane. The spongy mesophyll (S) is centric, i.e. palisade cells (P) line both upper and lower epidermis. In the section shown there are 8 vascular bundles (v) running parallel to each other, the one in the midrib region being the largest. Each bundle is surrounded by cells with brown contents (T), probably tannin, which is known to occur round the veins of Limonium binervum (Metcalfe and Chalk) another member of the Plumbaginaceae. It appears that each gland is located above or below a vascular element, and that the tissue density is slightly greater between a gland and a bundle than, elsewhere between bundle and epidermis. It is possible that the palisade cells at C are forming a connection between the gland and adjacent bundle. This is,

Plate 4

A



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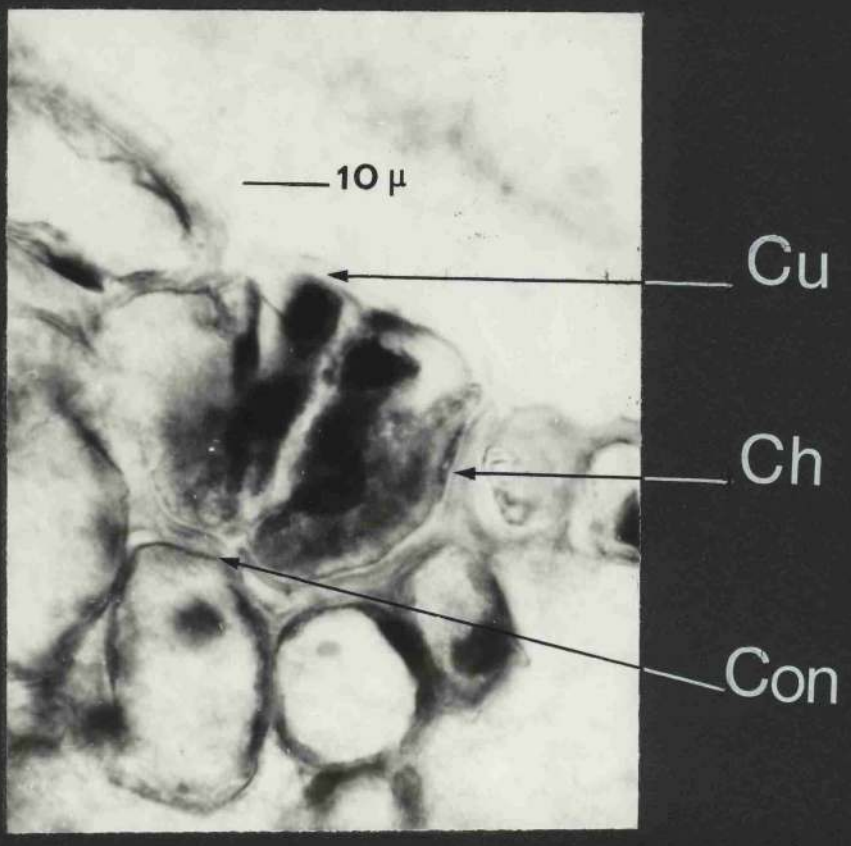
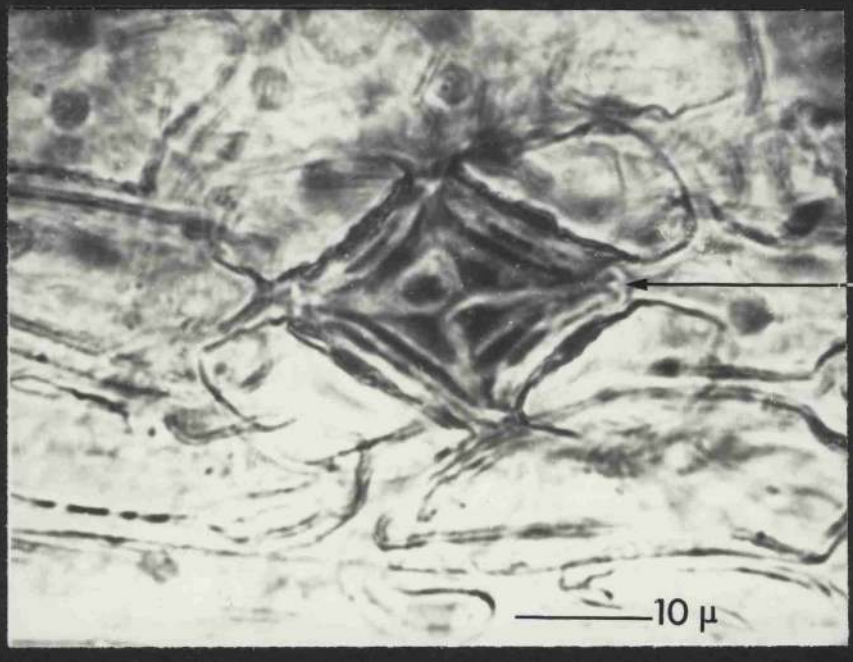


Plate 5



however, by no means certain as some cells in the chain may be out of the plane of the section.

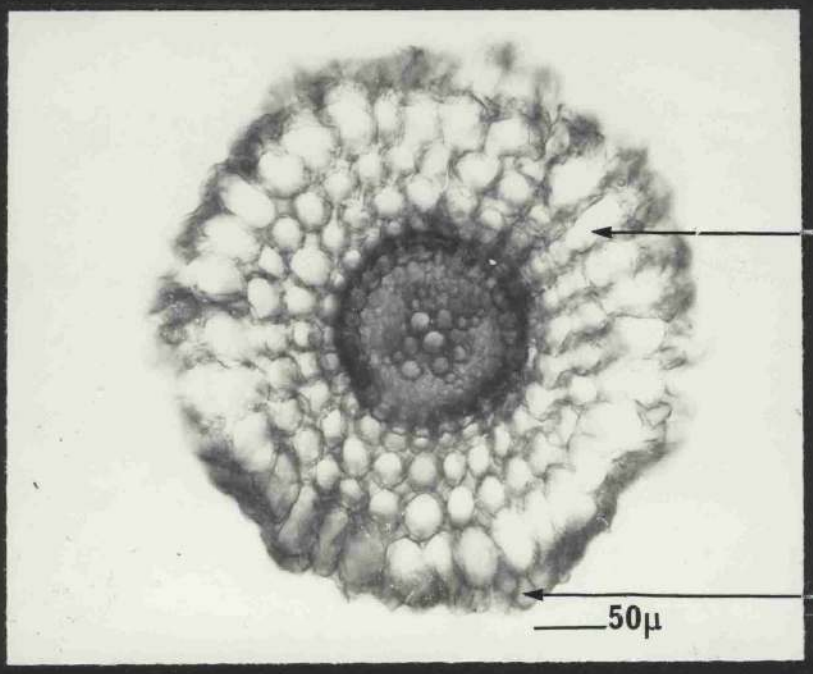
Plate 4 A & B shows high magnification photographs of the gland itself. The glands are conical and 40μ in diameter and made up of 4 large central cells (1), 4 smaller central cells (2), and 4 subsidiary cells (3), only 2 of each being visible in the section. The glands are covered to the outside by a cuticle (Cu), and are separated from the underlying cells by a distinct channel (Ch). Staining of sections with ammoniacal basic fuchsin alone (Gurr.E. 1965) showed that this channel was also filled with cutin. In A, a large space (5), is visible at the base of the gland with what appear to be the bases of 2 cellular connections radiating from it. Close observation of B shows the same space, but with distinct connection (Con) visible between the underlying palisade cell and this sub-glandular space.

Definite intercellular connections occur between the subsidiary cells and the epidermis. Plate 5 is a high magnification of the inside of a piece of epidermis stripped from a fresh leaf, which shows these connections (Con) quite clearly.

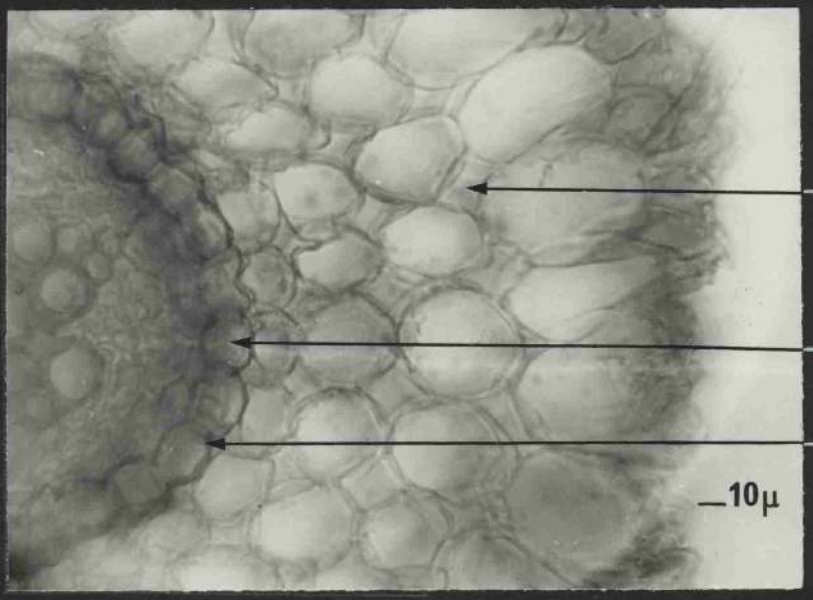
Study of the leaf anatomy thus indicated the occurrence of anatomical structures which could possibly play a part in ion regulation in the plant. These structures being small conical glands present in both the upper and lower epidermis, possessing

Plate 6

A



B



no physical connections with neighbouring cells, and possibly through the palisade tissue with the vascular system.

Roots

Both estuarine and montane Armeria maritima possess large root systems, based on a long top root with numerous branches. When grown in culture the older parts of the root are woody, and have heavily suberised peripheral layers appearing brown to the naked eye. The younger parts of the root, presumably the absorbing region, are white, and covered in the case of estuarine Armeria with numerous fine root hairs. Root hairs are present in montane root systems but to a very much lesser extent.

Attempts were made to fix and stain the young roots by the methods adopted with the leaves. These attempts invariably resulted in total collapse of the cortex leaving the stele surrounded by a heavily suberised endodermis.

Hand sections were made which were stained in safranin in 50% alcohol. Although the structure of the cell walls was slightly affected by the dehydration, the overall relationships of cells in the root can be seen in plate 6. A is a low power photograph of the root and shows the relative proportions of stele and cortex. The xylem is quadrarch and the endodermis prominent. The outermost continuous layer of cells is the exodermis (Ex), but the remains of the piliferous layer (P) can be seen. The radial

files of cells are obvious, and in B the many large intercellular spaces (I) can be seen. The innermost cortical layer has become slightly squashed against the endodermis (End), which clearly possesses thickening (T), indicated by the greater density of safranin staining, even in this young root.

Dehydration has reduced the size of the intercellular spaces relative to their size in the freshly cut sections, but these spaces are still prominent. The thickening at the endodermis is slight in comparison with sections of older roots, but its presence in this young tissue may be indicative of the site of a mechanism of ion regulation. The large exodermal cells are tightly packed along their radial walls, but at this stage in their development suberisation, which is present in older sections, is not apparent.

Section 3.

Obtaining ionic samples representative of xylem sap

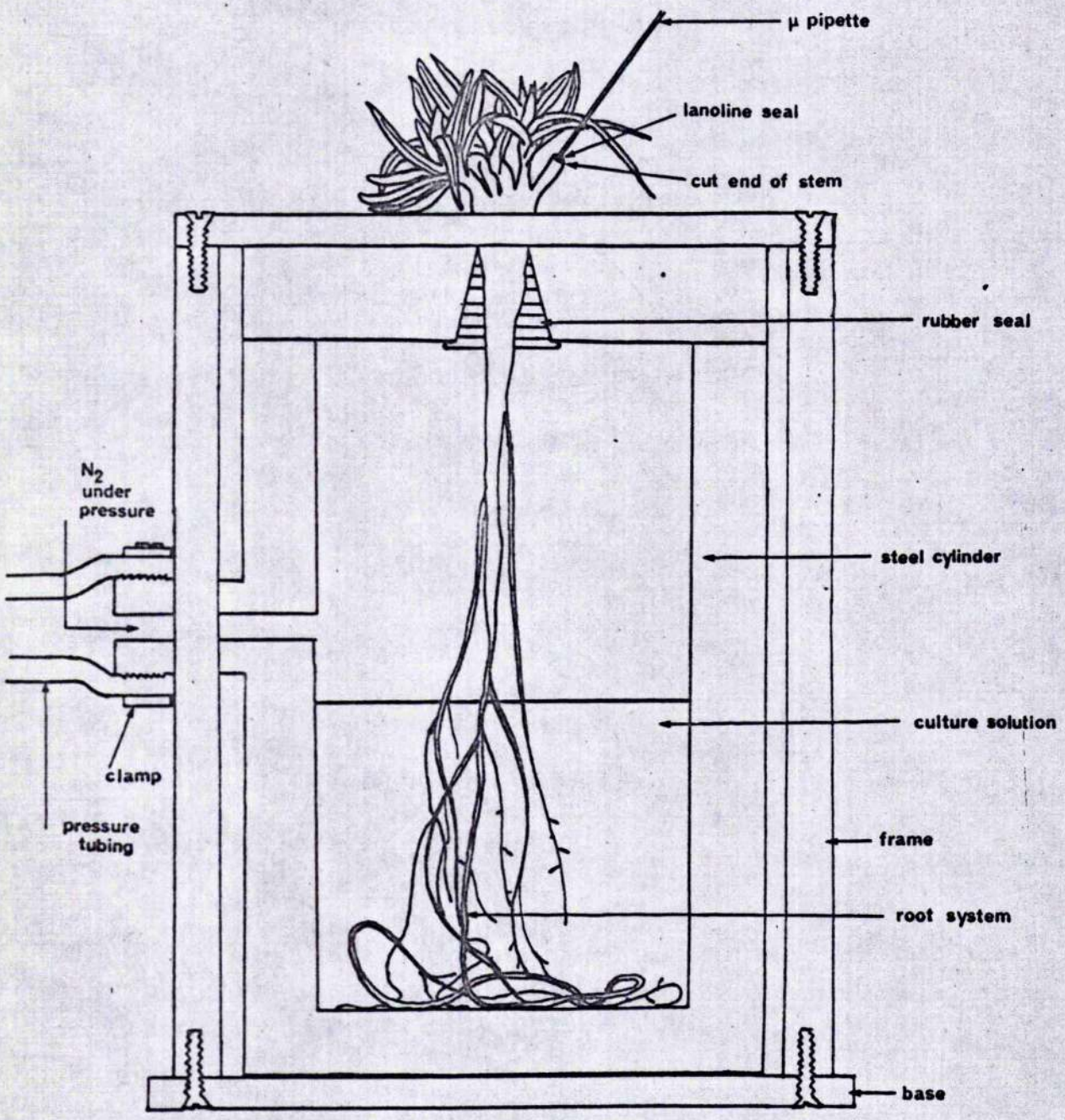
Two methods of obtaining sap were tried using Zea mays as the experimental plant, the Armeria being too precious to sacrifice unnecessarily. In both cases measures were taken to induce root pressure by enclèsing the plants in an artificially humid atmosphere under a high illumination. The trays of maize were enclosed in a large polythene bag supported by a wire framework, the inner surface of the bag being liberally sprinkled with water. The illumination was provided by 2 quartz-iodine 250w lamps fitted with heat filters.

The first method tried was that of vertical displacement. A cut piece of stem was clamped vertically above a micropipette, and a solution of congo red was applied to the upper surface to displace sap from the xylem vessels. True xylem sap would remain free from dye, and the end point of displacement would be seen to have been reached when dye began to come through.

The amount of sap obtained by this method from 10 cms of stem was negligible, and the likelihood was that even less would be obtained from the short, woody stem of Armeria.

The second method consisted of an attempt to blow sap from the stem by applying air under pressure to the morphological base, and having a micropipette at the morphological apex to collect

Figure 1


μ pipette
lanoline seal
cut end of stem
rubber seal
N_2 under pressure
steel cylinder
culture solution
clamp
pressure tubing
frame
root system
base

sap. The system was sealed with a 95% lanolin/5% paraffin mixture. This also failed, and as congo red solution could be blown through and collected, obviously the root pressure was not stimulated sufficiently to overcome the water tension in the xylem vessels, likely to be appreciably less in Zea than in Ameria.

The next system tried was that used so successfully by Anderson and Reilly (1968) and House and Findlay (1966). About 10 cms of top root were removed from an Ameria plant, and its cut surface sealed into a 50 µl 'Microcap' pipette using 95% lanolin/5% paraffin as a seal. The idea was that with the root still being immersed in aerated culture solution any exudate from the stump could be collected. This system could also allow a measurement of E.M.F. between ecudate and solution.

However, no exudation occurred after a period of 72 hours.

It was decided to construct an apparatus incorporating features of those designed by Jackson and Weatherley (1962), and Scholander et. al. (1965), in order to apply pressures of up to 30 atmospheres on the roots and their bathing solution (Figure 1). This application of pressure was to have caused inward and upward flow of water and mineral nutrients, by overcoming the negative pressure in the xylem. A sample of exudate could then be collected in a micropipette at the cut stem surface.

Unfortunately, a seal good enough to withstand the high pressure could not be produced, the system leaking rapidly when subjected to pressures in excess of 5 atmospheres. The plant, with its much-branching and tapering shoot proved difficult to accommodate in the rubber collar, and was often completely displaced from the system. This tended to prove injurious to the plant, and so it was decided to try an indirect method while material remained available.

The method chosen was that of elution of ions from leaves which had been cut into 1 cm. pieces, and then cut along the midrib to within 2 mms. of one end. It was hoped that by following the progress total of ions eluted with time, it would be possible to ascertain that time necessary for ions to be eluted from the extra-cellular spaces of the leaf.

If it is assumed that all cells in the treated leaf are equally available to the eluant, owing to the large inter-cellular spaces of the leaf and the dissection treatment, ions will be eluted from each cell in parallel. Thus the effect observed will be of elution of ions from a tissue behaving as though the tissue were composed of one large cell. Damage to the living tissue caused by cutting is likely to be small, as the longitudinal cuts were made along the midrib, and hence along the main vascular bundle, containing a large amount of xylem. Damage would be expected to result in error at the start of the

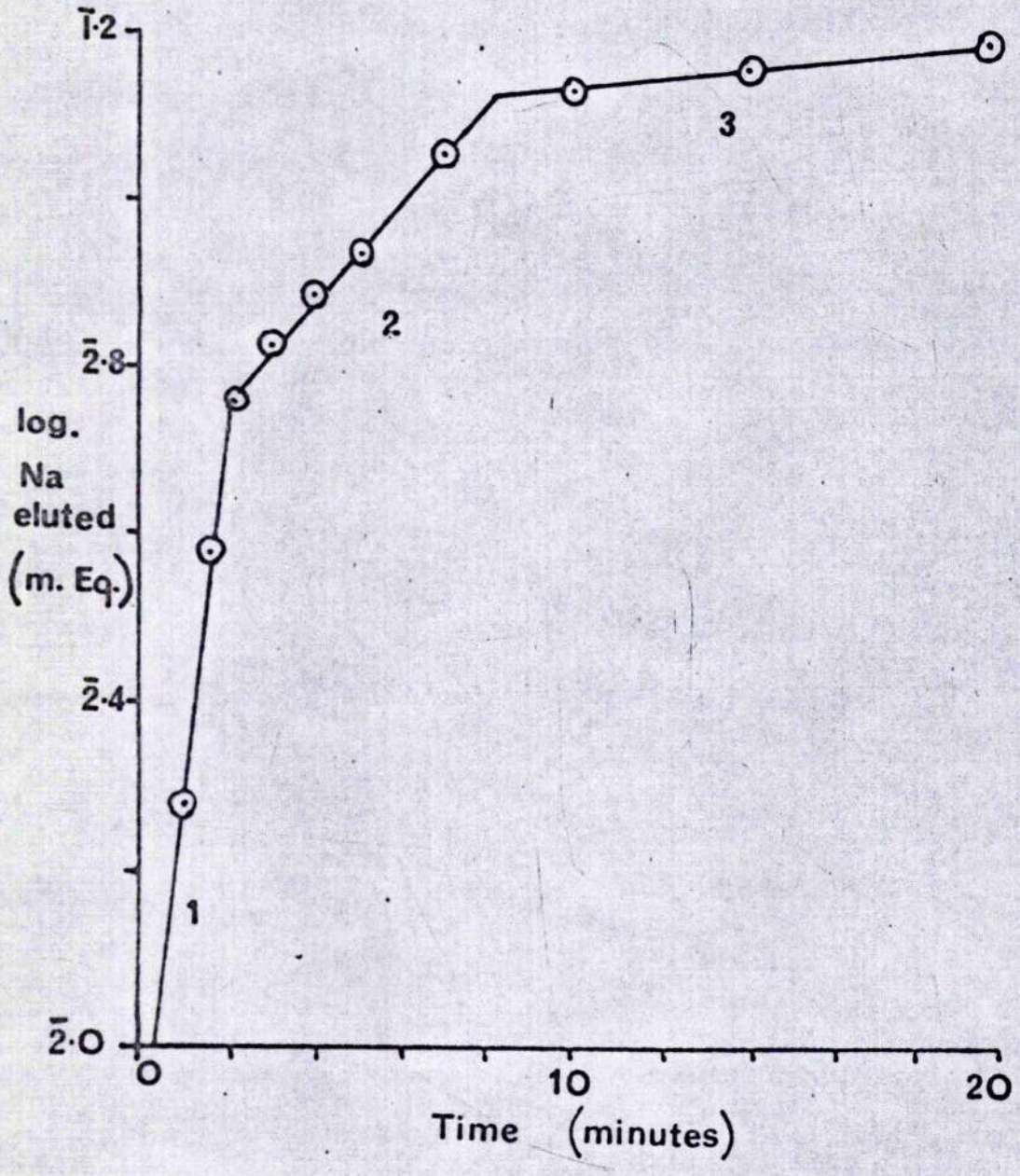
experiment when ions were eluted from cells which have been cut and were previously living. As the number of damaged cells is liable to be small in relation to the number not damaged and each cell contributes only a fraction to the total amount of ion eluted, the damage may have only a negligible effect on the elution curves.

If the protoplasmic permeability barriers are effective, then ions to the outside, in the intracellular spaces of the leaf will be eluted more rapidly than those within the protoplast. Thus a logarithmic time course of elution will show a break or inflection, if elution from the free space is so near completion as to be slower than elution from the protoplast. Using this break it should be possible to estimate the quantity of each ion present in the free space and protoplast respectively. If at each moment the rate of ion elution from each tissue phase, is proportional to the quantity of ions still remaining to be eluted from it, then the rate of elution will decrease exponentially with time. This leads to a linear relation for each phase, between time and the logarithmic value of the amounts of ions eluted, exposing any point of inflection clearly.

The elutions were performed in the following manner: 3 mls of distilled water were pipetted into each of 11 clear polythene phials, and a 100 ml. sample of the same water set aside

Figure 2

Log. progress total Na eluted from leaf



for use as a standard in flame photometry, and to dilute the eluant for use in chloride estimation. Several mature leaves were cut transversely into 1 centimetre sections, and each section was slit longitudinally along the midrib to within 2 mms. of one end. The sections were threaded in turn onto a fine piece of copper wire.

The threaded sections were then suspended successively in the 11 phials, the time in each varying, being longer as the elution progressed, the total time being 19 minutes. The solutions in each phial was stirred continuously when the elution was taking place, by agitating the wire to which the leaves were attached.

Figure 2 shows a typical log. plot of the progress total of the eluted ions. The concentration quoted refers to the concentration in the phials, not the concentration in the leaves. It can be seen that Na^+ was eluted fastest in the first 2 minutes, and that this phase of rapid elution was followed by 2 successively slower phases. Similar graphical representation of K^+ and Cl^- results showed the same 3-phase effect, differing only in that the length of phase 2 was 40 seconds shorter in the case of Cl^-, and the slope of phase 3, which was appreciably smaller in the case of Cl^-.

These results show the presence of 2 inflection points, so the elutions did not draw a firm delineation between ions from the free space, and ions from the protoplast as hoped. The phases

observed had to be related to the morphology of the leaf, in particular to the A.F.S. (apparent free space) and the A.O.V. (apparent osmotic volume), and to the possible effects of damage.

The free space of any tissue has been defined by Briggs and Robertson (1957) as "that part into and through which the solute and solvent move readily, compared with the part of the cell or tissue between which and the external solution, the solvent, but not the solute exchanges readily - the osmotic volume". The apparent free space is the apparent volume of the free space, and similarly the apparent osmotic volume is the apparent volume of the osmotic volume.

The elution curves could be interpreted in three ways. Phase 1 may have been the result of elution from the wet film on the cell walls and sap-injected intercellular spaces plus damaged protoplasts, whilst phase 2 consisted of elution from the A.F.S. in the cell walls, and phase 3 of elution from the protoplast. Elution from the protoplast would be controlled at the plasmalemma. Alternatively, phase 1 was the complete elution from the A.F.S. and damaged cells, phase 2 from the cytoplasm, and phase 3 from the vacuole. The third possibility was of successive elution from damaged cells, A.F.S.; and protoplast.

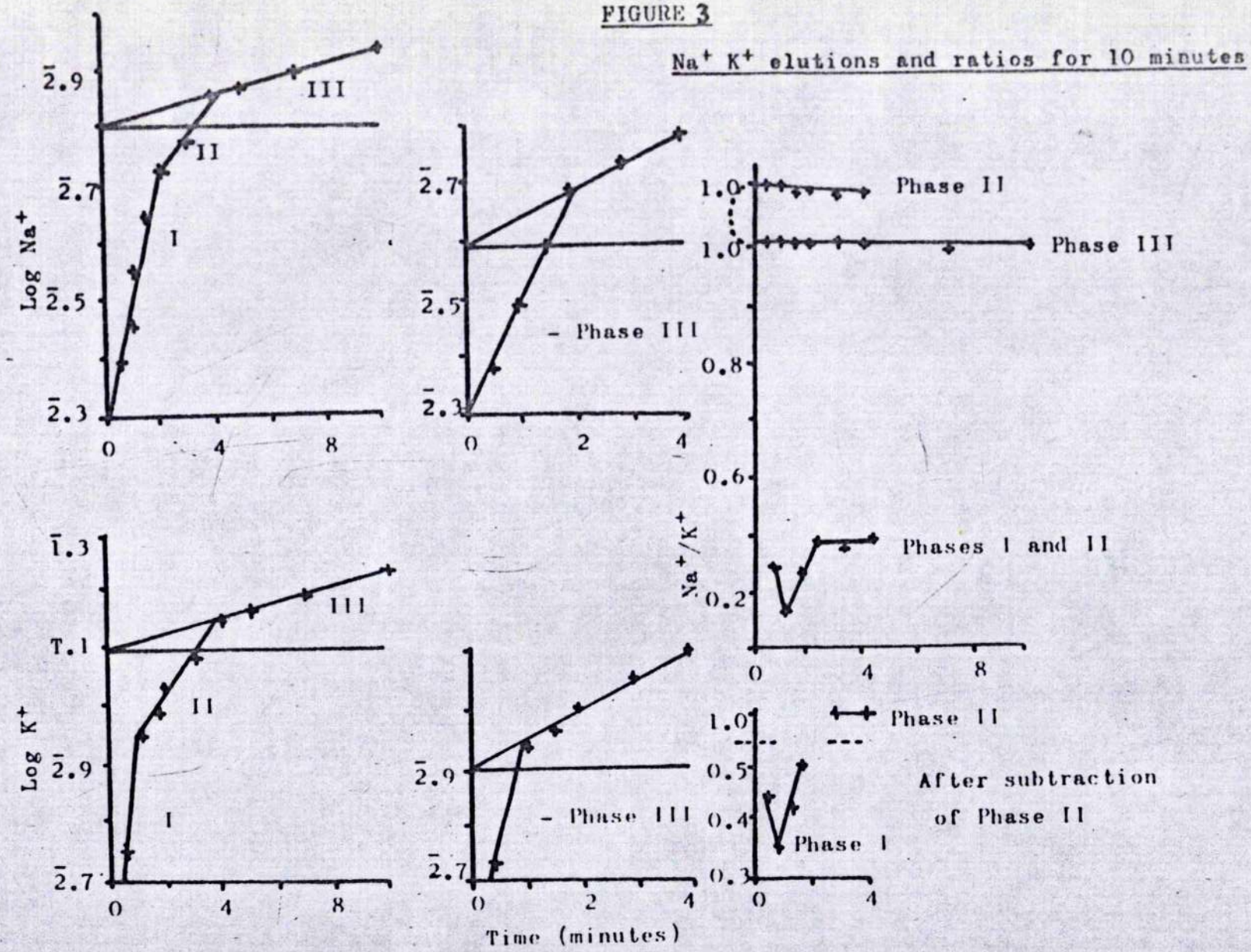
These hypothesis could be tested relatively easily. As

the A.F.S. in the leaf is a continuous system, ions present in the water film in the free space, (the W.F.S. or water free space) will be at the same concentration throughout the system, and in all probability at a different concentration from ions in the A.O.V., and the rates of elution will be proportional to these concentrations. Thus if the ratios of Na:K were plotted throughout the time course, a change in the value of the slope would indicate a change of concentration of 1 or both ions in the phase from which they were being eluted, as compared with the preceding phase. That is, if both phases 1 and 2 were components of the A.F.S., the concentration of both ions would be the same, and the possible physical barrier of the cell walls would not be manifest in a log. time course of the ratio of the ions eluted. It is probable that the three phases are in series and in equilibrium with each other in the leaf in situ. Exposure of the treated leaf to the water causes rapid loss of ions from phase 1, ad will upset the balance of this equilibrium. Thus phase 2 will lose ions to phase 1 and phase 3 to phase 2. Alternatively the three phases may not behave in this manner, phase 1 emptying before phase 2, and phase 2 before phase 3.

The former was considered probable and so corrections were made in an attempt to allow concurrent loss from each compartment, prior to plotting change in Na:K with time.

FIGURE 3

Na^+ K^+ elutions and ratios for 10 minutes



Loss of ions from phase 1 was rapid and was likely to result in an immediate loss from phase 2. However, the rate of loss from this latter phase was slower and may not have had an immediate effect on phase 3. It was proposed that assuming loss of ions from the 3 phases began simultaneously, or almost so, that subtraction of the slowest phase (3) from the second; followed by the slower two from the fastest, would yield more accurate determination of the rate of ion loss from the individual compartments.

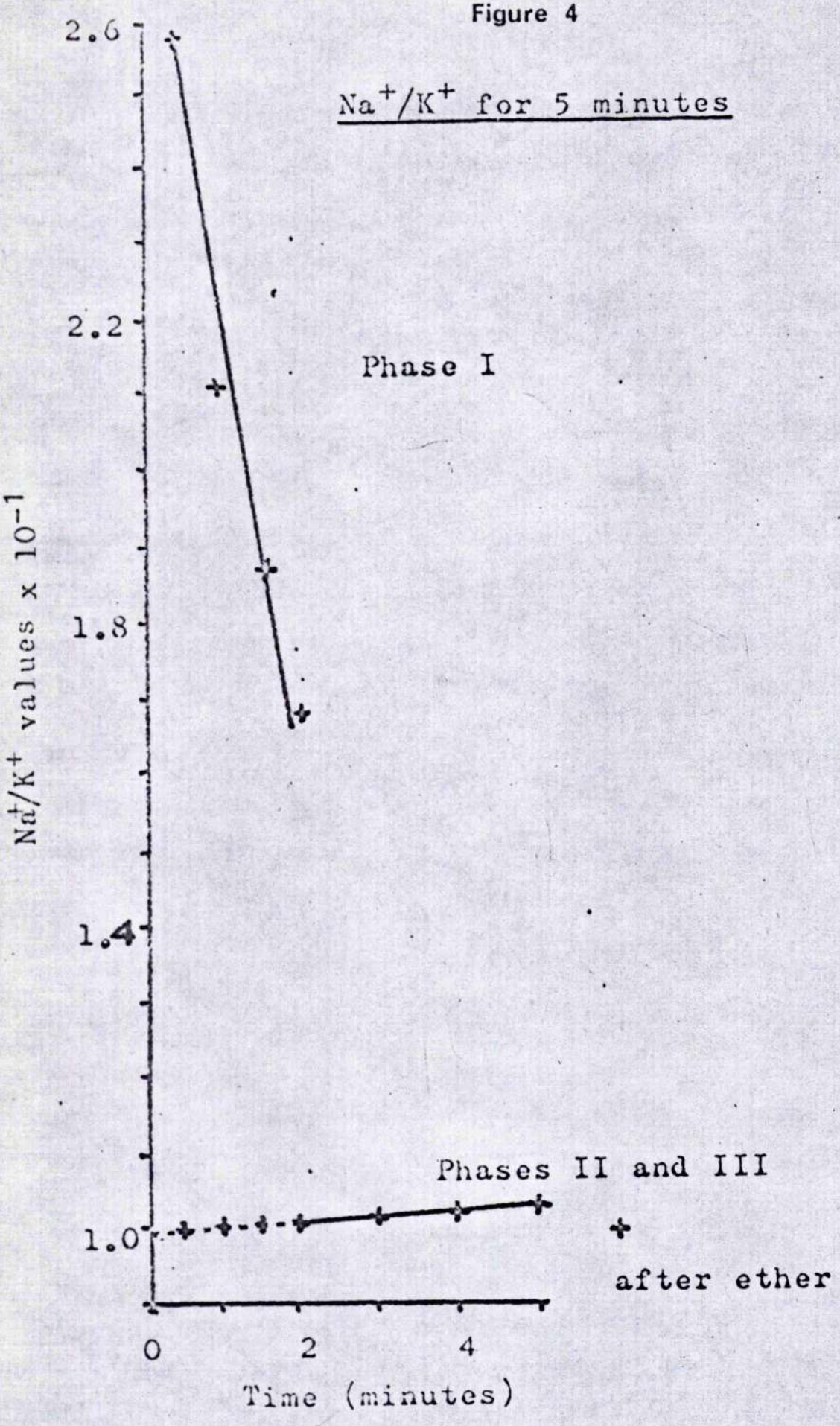
Ratios of Na:K in the eluant with increase in time were obtained in this manner following a 10 minute elution, and are presented in Figure 3.

Ratios for compartments 2 and 3 have been extrapolated back to zero following, in this figure, the assumption that elution is proceeding concurrently in all 3 compartments.

The values for Na:K in phases 2 and 3 are unity, but phase 1 shows an early inflection. This may have been caused by contamination from damaged protoplasts or be the result of subtraction of estimated phase 3 elution which had not begun immediately the leaves were immersed in water.

A further elution for five minutes only was performed, so that no values for elution from phase 3 when this phase is rate-controlling were obtained. The results after subtraction of phase 2 are shown in Figure 4, and the value of unity for

Figure 4


Na^+/K^+ for 5 minutes
2.6
2.2
1.8
1.4
1.0
Phase I
Phases II and III
after ether
Na^+/K^+ values x 10^{-1}
0
2
4
Time (minutes)

phase 2 was confirmed. No reflection point occurred. The tabulated value 'after ether' refers to the Na:K ratio, after 18 hours in ether, which destroys plant membranes and hence allows free elution from the protoplast. A value of unity was observed confirming previous data.

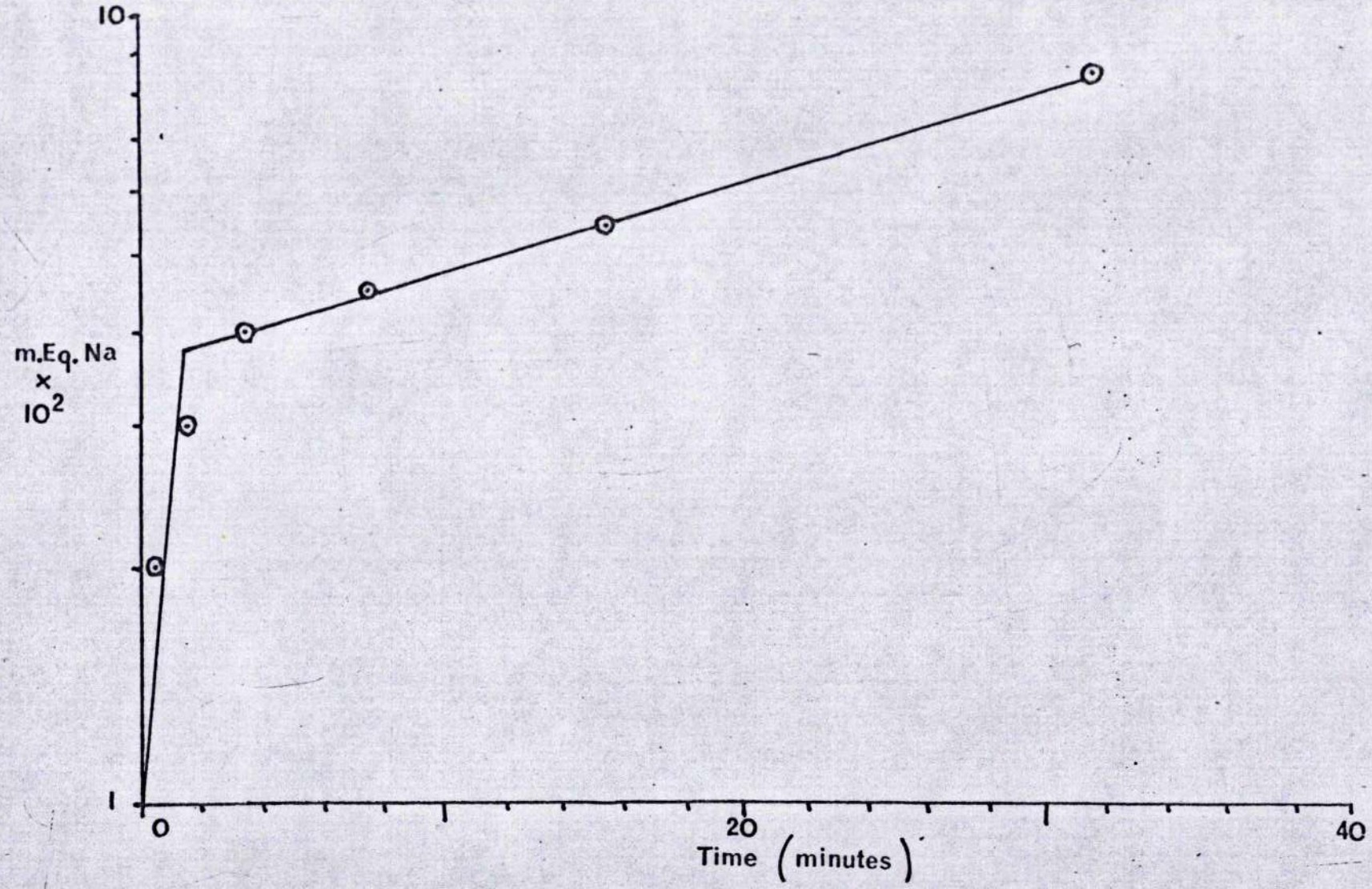
The absence of the inflection point in phase 1 may be indicative of no contribution by phase 3 ions to the elution immediately after commencement, or it may be the result of greater care in dissection, or greater analytical accuracy caused by a greater quantity of eluted ions. At low concentrations the analytical procedures become less accurate with the equipment used (Section 4).

However results indicate a discontinuity between phases 1 and 2. This was attributable to the existence of a barrier, the plasmalemma, separating phase 1, the A.F.S., from phase 2, the cytoplasm.

As had been mentioned above, ether destroys membrane semi-permeability. An experiment was performed to ascertain whether discontinuity still existed between phases 1 and 2 and 2 and 3, during elution in ether. Effective loss of a permeability barrier should result in a constant rate of elution from within a membrane bounded system after the bounding membrane has been destroyed, but not at the outermost barrier.

Figure 5

Log. progress total Na eluted into ether



The results are plotted in figure 5. The phase boundaries are not so distinct between 1 and 2, and non-existent between 2 and 3. Phase 1 is slightly shorter, and the gradient of phase 2, steeper. This was considered to be indicative of the increase in membrane permeability resulting in rapid ion loss from the protoplast, which masked the later stages of phase 1. This is evidence in itself that elution from phases 2 and 3 does not begin concurrently with phase 1 in a normal elution. There is a slight time lag before loss of ions from phase 1 shifts the equilibrium state of the 3 phases towards loss of ions from the inner compartments. Loss of distinction between phases 2 and 3 confirms the presence of an ether sensitive barrier between these phases.

It was concluded that phase 1 consisted of elution of ions from the A.F.S. of the leaf, and possibly of some contamination from damaged cells, and that phases 2 and 3 were elution from cytoplasm and vacuole respectively.

Therefore it was postulated that use of a 2 minute eluant would provide an ionic sample representative of xylem sap, with little contamination from other compartments. The concentration of these ions would be known after the volume occupied by them in the leaf had been determined.

The compact root structure precluded use of this approach for measuring ionic concentration in the root compartments.

Section 4.

Analysis of ions in eluant

The cations, Na and K were assayed using an 'Eel' flame photometer. Readings obtained were converted to milli-equivalents (mEq/l) by comparison with standard calibration curves. These curves were constructed from mixed salt solutions to eliminate possible interference of 1 ion with analysis for the other. The range of concentrations used was 0.01-2 mEq/l.

Analyses for Ca^{++} by the flame photometric method of Derderian (1961) and by the spectrophotometric methods of Herrero-Lancia and West (1963), failed to yield satisfactory results at the low Ca^{++} concentrations necessary.

Cation assay was limited to analysis for Na and K because; these are the most important cations in a study of salt regulation; and Ca was only present in the eluant at very low concentrations and could only be assayed using sophisticated techniques. Experimental work envisaged, would lead to much routine measurement of concentration, which must be kept as simple as possible within the limits of experimental error. Samples to be analysed were of small volume, 5 mls., and removal of large quantities of this sample, for analysis of ions other than those envisaged as playing a major part in the research,

could not be justified.

The anion, Cl, was assayed using an 'Eel' chloride meter. Cl^- reacts with Ag^+ electrolysed from an Ag wire at constant rate. This reaction proceeds until the Ag^+ concentration exceeds that of Cl^-, when the potential of the Ag electrode changes steeply with respect to a reference electrode, causing cessation of electrolysis. The period of steady electrolysis is proportional to the initial Cl^- content of the sample.

3 mls of each 5 ml sample were diluted to 10 mls with distilled water, and 3 mls of acid buffer and 5 drops of thymol blue/agar were added. These solutions were made up according to the manufacturer's instructions.

The reading given by the meter at the end point of electrolysis, was converted mEq/l by a simple proportion calculation after subtraction of a 'blank' reading, obtained using 10 mls of distilled water.

Assay for $SO_4^=$ using an 'Eel' photometer to titrate 1 ml of sample against 0.01M $Ba(ClO_4)_2$, with 3 drops of B.D.H. Sulphonazo III (0.1%) as an indicator did not give reliable results at the low dilutions found. Incorporation of analysis for $SO_4^=$ in the main experimental procedures was, therefore, rejected on the grounds listed above for Ca^{++}, since $SO_4^=$ was not a major solute ion in the tissues.

Section 5.

Methods employed to find the volume occupied in the leaf by ions in the phase I eluant

It has been established that the ions eluted in Phase I came from that part of the Apparent Free Space (A.F.S.) known as the Water Free Space (W.F.S.). This, then, is the volume which it was necessary to measure in order that the concentration of ions inside this space be known.

The method would have to be used as a routine part of a series of experiments, in which the effect on electric potential, and internal ionic concentrations, of changing the concentrations and proportions of ions in the bathing medium, was studied. The method would, therefore, have to be both simple, and have an accuracy of result within the bounds of other experimental error. This simplicity ruled out any isotopic method as used by Briggs, Hope and Pitman (1958), Bernstein and Nieman (1959) on plants, and Goodford (1961) on animal tissues.

An obvious choice was a method based on that of Briggs, Hope and Robertson (1961).

Leaves were cut and threaded on a length of wire as described in Section 3, and then immersed in water in a beaker located in the chamber of a vacuum dessicator, the vacuum being

released several times, until all the intercellular spaces had been injected with water. If the weight before and after injection is noted, the volume of the injected intercellular spaces can be calculated. This volume must be known as it is part of the A.F.S., but must be subtracted to give the volume of the normal ion-containing W.F.S., Before weighing on the Mettler electric balance the leaves had to be blotted to remove a surface film of water. The injected leaves were then weighed, immersed in water, in a beaker sitting on a wooden bridge, and transferred to a beaker of 0.3M mannitol. The leaves and wire were submerged to the same depth in each solution. On transfer to the mannitol solution, the leaves appear to gain in weight, as mannitol displaces water from the vacuum-filled spaces of the leaves, and the leaves settle into the mannitol. This gradual increase in weight should halt when mannitol has permeated fully into the extra-cellular spaces.

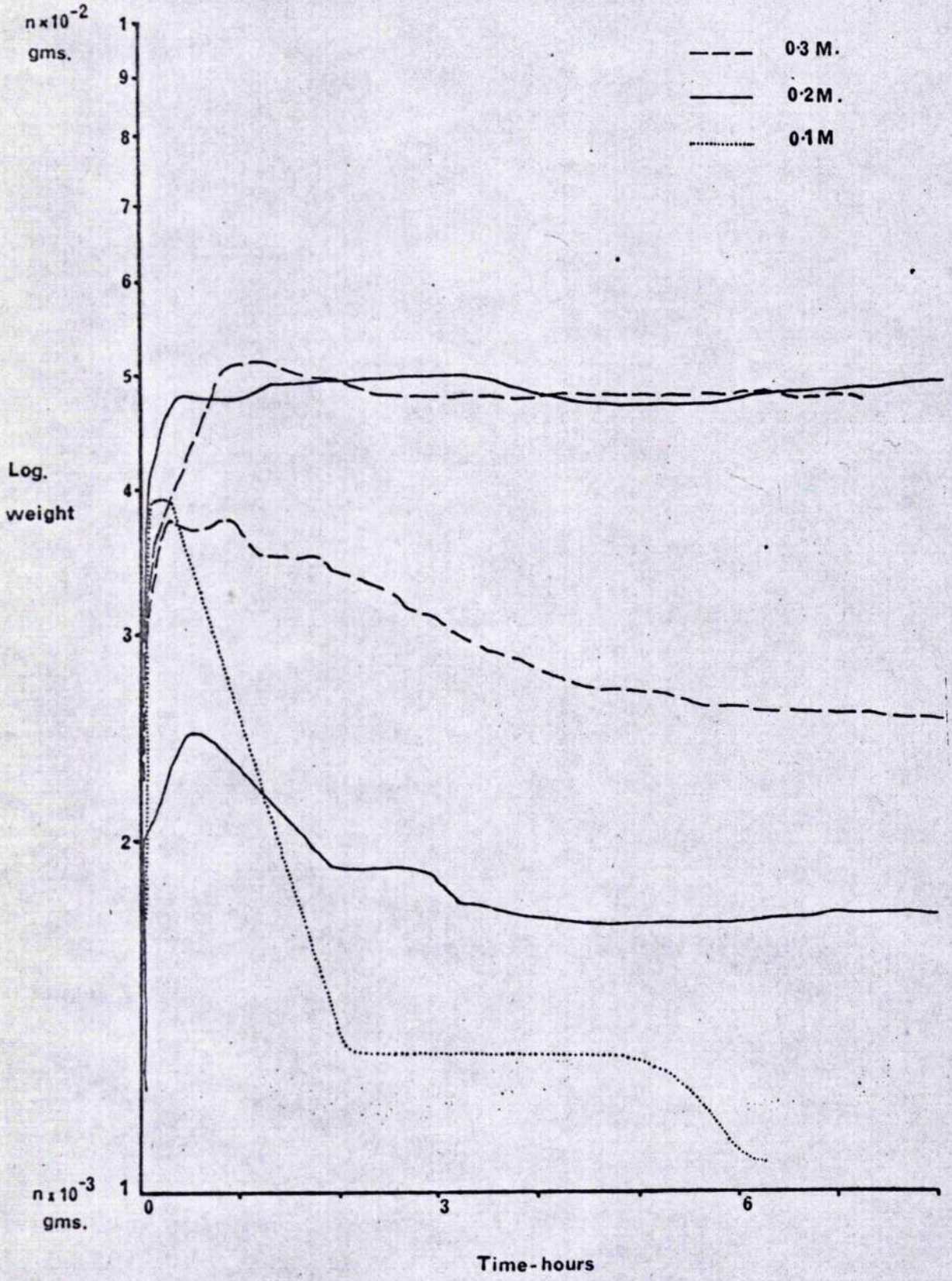
The leaves were then removed from the wire, blotted, and weighed in air (Wa). The wire was weighed in water and mannitol without the leaves, these values being subtracted from those already obtained from the leaves plus wire in the same liquids, to give Ww and Wm, for water and mannitol respectively. The densities, Pw for water, and Pm for mannitol, having been determined, the A.F.S. was calculated as follows:-

$$V = \frac{(Wa - Wm)}{Pm}$$

where V is the volume of tissue in mannitol - i.e. the weight of

Figure 6

Weight of water-impregnated leaves in differing concentrations of mannitol



the displaced mannitol. The A.O.V. - the Apparent Osmotic Volume, the protoplast volume not penetrated by mannitol was:-

$$A.O.V. = \frac{(Ww - Wm)}{(Pm - Pw)} \text{ and hence the A.F.S.} = V - A.O.V.$$

Values obtained using this method were too small and too variable to be of practical use. However, as an approach along these lines seemed to be the only possibility the steps of this method were analysed, and various modifications attempted.

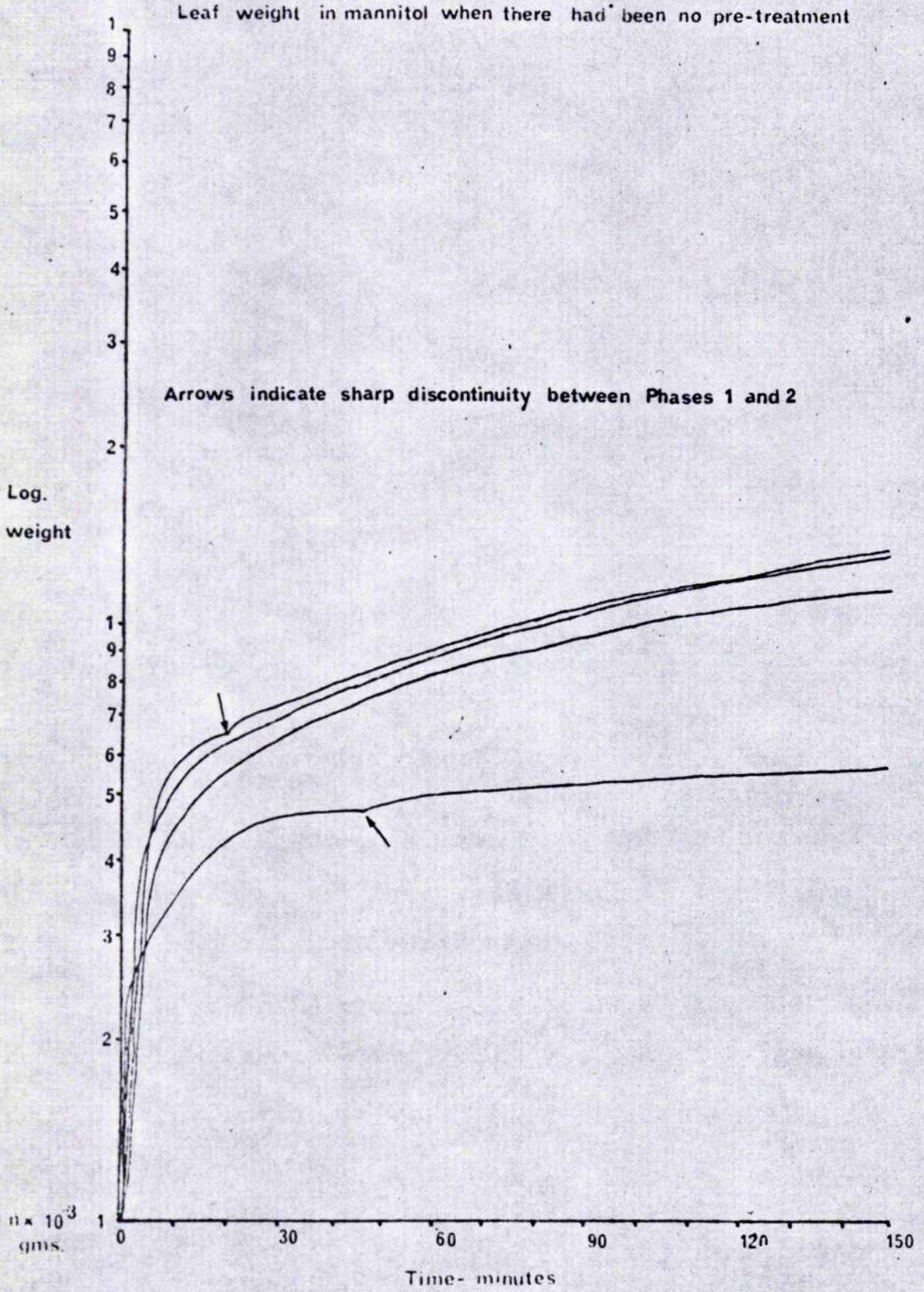
(i) Increase in leaf weight in mannitol

The first problem was to find the time taken for mannitol to occupy the A.F.S., and so a series of time courses of increase in weight in mannitol, of molarities differing along a range of 0.1-0.5M, were performed using leaves which had been previously impregnated with water. Several of these time courses plotted logarithmically are shown in Figure 6. It should be stated that readings for this series of graphs were taken at intervals of 1 minute, and so individual points are not recorded on the graphs.

The time courses show an initial increase in weight, followed by a decrease which was sharp or gradual. These results seem to indicate that loss of turgor, with or without reversible plasmolysis, was occurring. Initially this was manifested by an increase in weight, as water exosmosed, followed by recovery of

Figure 7

Leaf weight in mannitol when there had been no pre-treatment



turgor as mannitol diffused slowly into the protoplast. As the cells recovered their turgidity, mannitol was displaced from the volume it had occupied following decrease in the protoplast volume. The leaves now became less dense, which is reflected by the decrease in weight in the time course.

Not all leaf samples followed the same behaviour; the steady values attained after 45 minutes possibly being the result of a high osmotic potential, in the protoplasts of the cells of these leaves. No direct visual evidence was recorded of plasmolysis at any of the mannitol concentrations used.

(ii) Effect of no water pre-treatment

Time courses were recorded for leaves which had not been subjected to prior impregnation of the extra-cellular spaces by water. Figure 7 shows a sharp increase in weight of these leaves, followed by a more gradual rise, which reached a plateau after 14-18 hours in mannitol. This figure, showing only the early stages of these time courses, contrasts with the sharp and gradual transitions from rapid to slow increase in weight, found with different leaf samples.

It would appear by contrasting (i) and (ii), that water pre-treatment had an effect, possibly on the membrane, causing turgor loss in some cases. The absence of water pre-treatment resulted in a 2-phase system, presumably caused by mannitol entry

Figure 8

Leaf weight in: a – mannitol. b – subsequent water after varying the duration of water pre-treatment

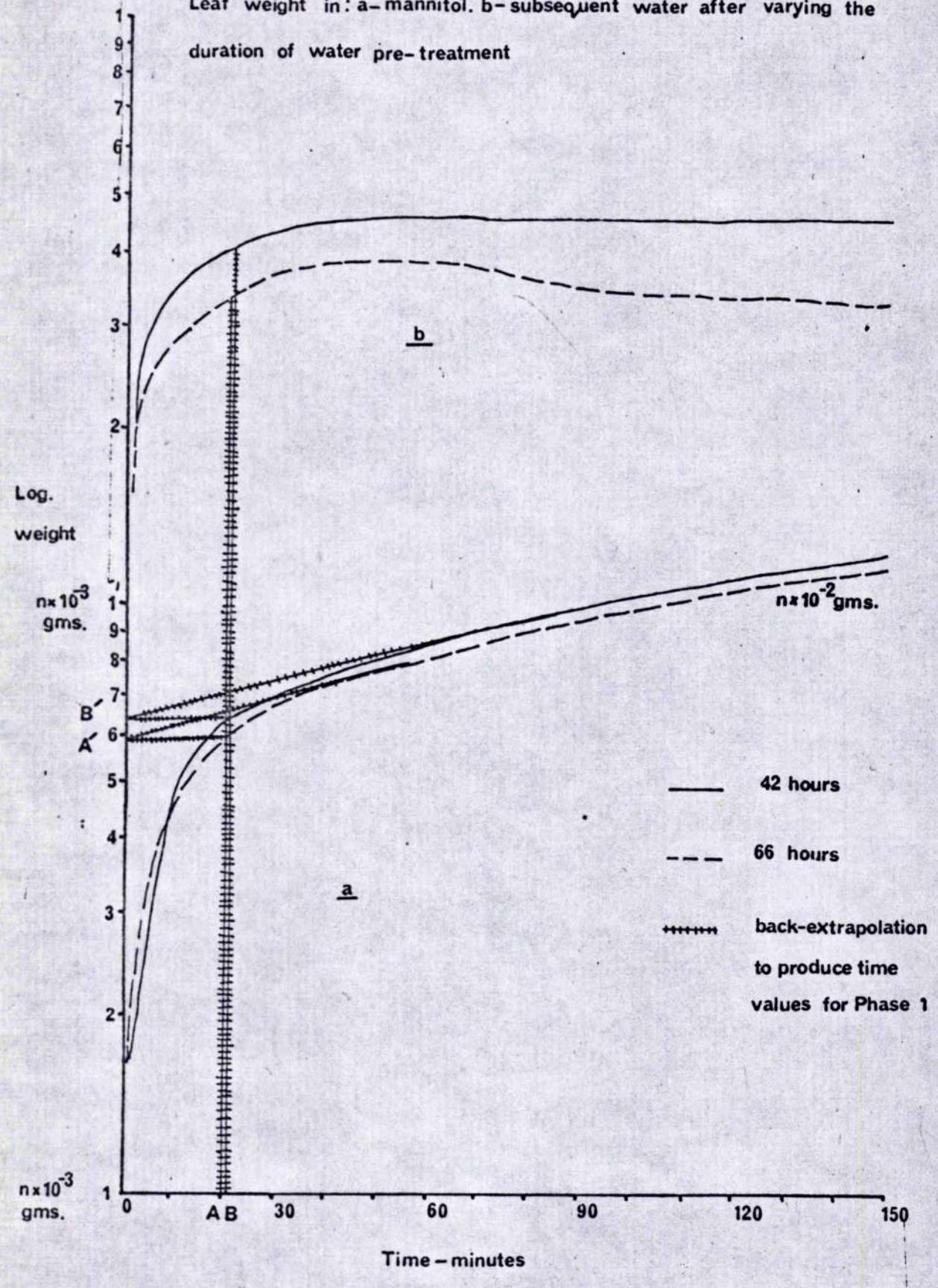
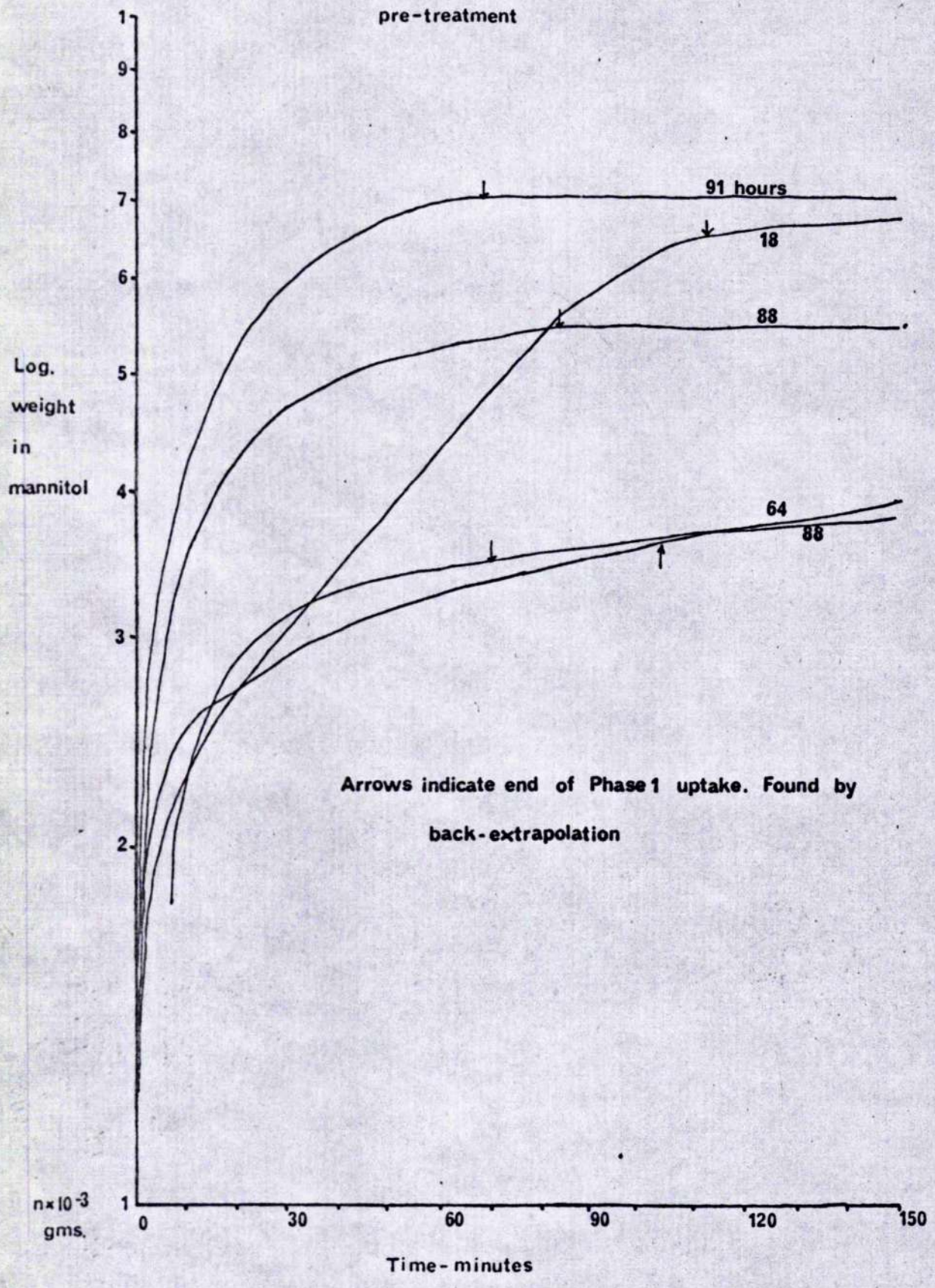


Figure 9

Leaf weight in mannitol after varying the duration of water pre-treatment



into 2 distinct leaf compartments.

The slope of phase 2 was constant, and the end-point of phase 1 was reached after 40 minutes.

(iii) Variation in the duration of water pre-treatment

Phase 2 was possibly attributable to uptake of mannitol through a membrane. If this were so, subsequent weighings in a less dense medium, following Briggs, Hope and Robertson's method, would be in error, owing to mannitol having accumulated in the cells. Hence it was proposed to study the effect of varying the duration of water pre-treatment, in the hope that a definite end-point would be obtained, attributable to displacement of water from the extra-cellular spaces. The change in weight on subsequent return to water was also followed, to ascertain the time for displacement of mannitol by water from the extra-cellular spaces.

It would seem that duration of pre-treatment affected the rate of penetration of the extra-cellular spaces, by mannitol, (Figures 8 & 9). The more rapid penetration, and hence increase in weight, was associated with the longer periods of pre-treatment. Figure 9 shows an apparent reduction in the slow second phase after long pre-treatment, presumably due to a decrease in the permeability of the membrane to mannitol, induced by the pre-treatment.

Figure 8 (b) shows the attainment of a steady value after

return to water, the time for which corresponded closely to the end-point of phase 1 increase in weight in mannitol (a), found by back-extrapolation. It would seem from this result that phase 1, the rapid phase, does correspond with mannitol penetration to the protoplast, phase 2 being the result of diffusion into the protoplast.

It was concluded on the basis of the above results, that a 90-hour pre-treatment allowed for complete, rapid penetration of mannitol to the protoplast, and little penetration into the protoplast (Figure 9). Accordingly, it was proposed that a 90-hour water pre-treatment followed by 75 minutes in mannitol, should be used in the determination of the volume of the A.F.S., using the method of Briggs et al.

(iv) Experimental testing of modified method

A series of experiments was planned along the lines of those of Bernstein and Nieman (1959), i.e. to find the molarity of mannitol at which the volume of the A.F.S. increased, the increase being caused by plasmolysis. This value would, therefore, give an indication of the molarity of the cell sap, which would only be approximate, because of prior leaching of mineral ions in the water pre-treatment. More important, the experiments would indicate whether the technique was reliable.

The results obtained indicated that the technique was at fault in at least 2 places. The values for A.O.V. (Apparent

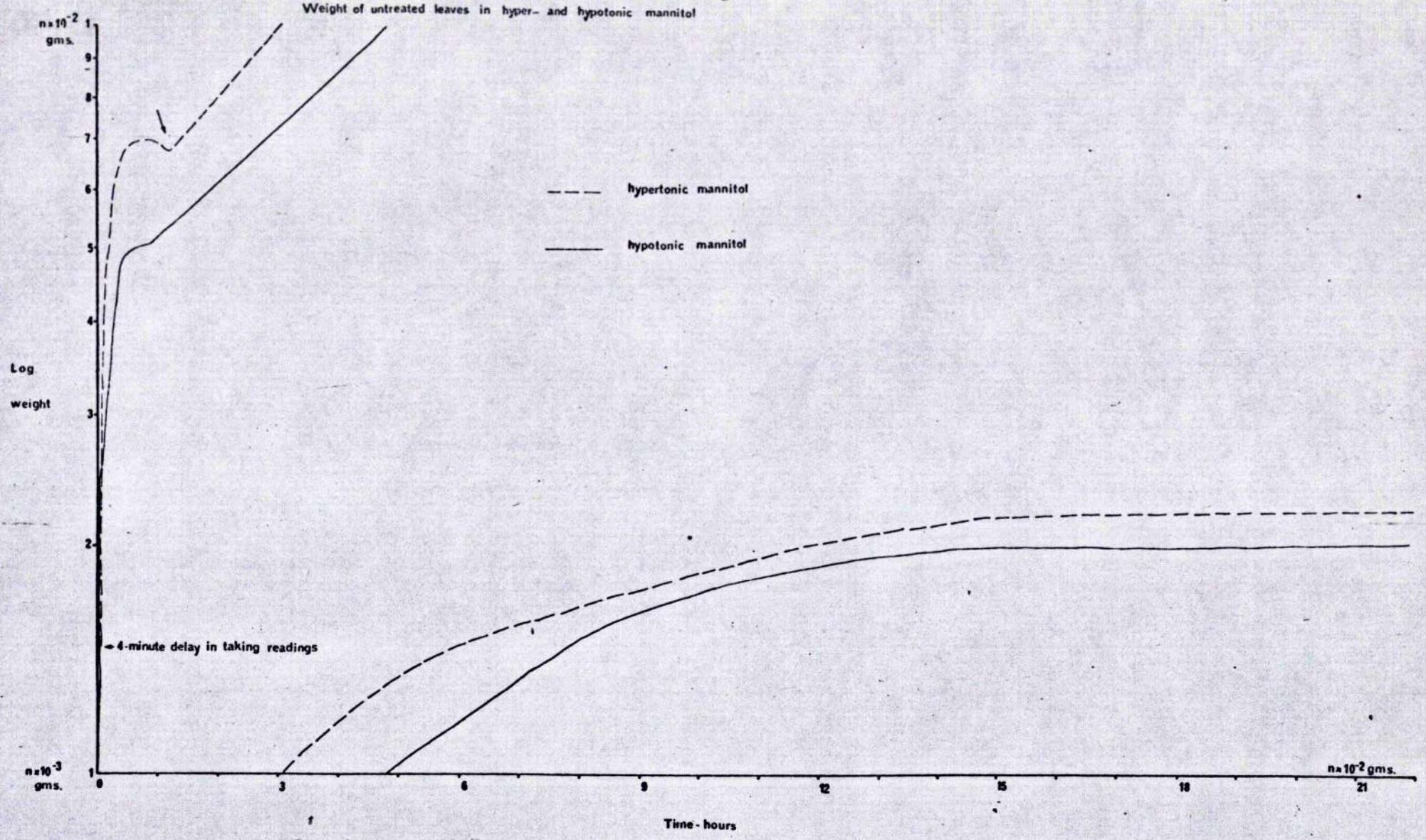
Osmotic Volume) were 1 order of magnitude too great, and often resulted in a negative A.F.S. It seemed that the plateau values (Figure 9) for mannitol uptake, were the exception, rather than the rule, for time courses plotted during the course of the test experiments showed no such tendency. There appeared to be no defined end point in time, to any phase of uptake, so values obtained for weight in mannitol, and weight of mannitol actually in the intercellular spaces, were erroneous. Other errors were incurred when blotting after water and mannitol treatment. This procedure had to be thorough but brief, and the subsequent weighing performed before loss in weight, due to evaporation from the micelles of the cell walls. Evaporation loss was reduced by weighing in a humid atmosphere, though care had to be taken to prevent condensation.

(v) Development of a new method for the determination of the volume of the A.F.S.

a) Identification of leaf compartments penetrated by solutions differing in density

The uptake of mannitol and paraffin by weighed leaves left overnight in the respective solutions was compared, the object being to see if the volume penetrated by the mannitol differed significantly from that by paraffin. The difference was significant, the mannitol penetrating 2.9 times the volume penetrated by the paraffin, after corrections for fresh weight and density differences.

Figure 10


Weight of untreated leaves in hyper- and hypotonic mannitol
n x 10⁻² gms.
hypertonic mannitol
hypotonic mannitol
Log weight
4-minute delay in taking readings
n x 10⁻³ gms.
n x 10⁻² gms.
Time - hours

The difference in weights, leading to the volume estimations, was too great to be accounted for by differences in the blotting treatment prior to weighing. Thus it appeared that at least 2 compartments of the leaf tissue were penetrated, i.e. either a membrane and the complete free space, or 2 components of the free space only.

In order to determine which of the above possibilities was in fact correct, time courses of increase in weight of untreated leaves in hypertonic mannitol were compared with those in hypotonic mannitol. The object was to see if there was any difference in either the duration or shape of the initial uptake phase. Such a difference could be attributed to the occurrence of plasmolysis.

The results are graphed in Figure 10. The broken curves are continued at the base of the graph because of ease of presentation on a single cycle plot. It can be seen that the two time courses correspond almost exactly, except that in the hypertonic solution phase 1 produced a greater increase in weight, due to the uptake of more mannitol by the leaves. The weights of leaves used in each part of the experiment were almost identical, and the material came from the same plant. Thus the difference observed must have been caused by plasmolysis.

It appeared that the first phase of the curves was concerned with uptake into the cell walls and intercellular spaces.

The 'hump' after 30 minutes was caused by a gradual permeation of mannitol causing de-plasmolysis bringing about a transient decrease in the volume permeated by the mannitol. As more penetration of the membrane occurred, the increase in weight caused by this overcame the reduction in free space caused by de-plasmolysis; and the leaves settled further into the medium.

The critical time for penetration of the free space by the mannitol appeared to be 35-40 minutes.

b) Use of solutions of differing density to find the volume of the A.F.S.

Having ascertained the time necessary for complete penetration by mannitol into the A.F.S., water and paraffin were compared as to their effects as second density media. It was hoped that one of these media would allow the volume of the entire A.F.S. to be calculated. For such a result, a time course would show an indication of a steady value, caused by the mannitol-impregnated leaf settling in the new medium, without displacement of mannitol from the tissue. Alternatively, displacement of mannitol from the large intercellular air spaces, by the second density medium, would enable a direct value for the volume of the W.F.S., and hence the ionic concentration of ions, to be calculated.

Accordingly, time courses were followed for leaves which had previously been immersed for 30 or 60 minutes in hypo- or hypertonic mannitol; the second density media being water or

paraffin (Figure 11).

It can be seen that there was a rapid increase in weight in both water and paraffin. However, the increase in weight in water was a smooth curve, whereas the time course of the leaves which had been immersed previously in hypotonic mannitol, had a distinct 'step', or discontinuity, of phases of 3-4 minutes duration, after 5 minutes. The large increase in weight after this 'step', was taken to indicate that paraffin was displacing mannitol from the free space.

Thus it appeared that the time taken for the leaf to 'settle' in the viscous paraffin was 5 minutes, and that 5 minutes later the paraffin began to displace mannitol from the A.F.S. The duration of this phase of displacement was ill-defined, but in the region of 15-20 hours.

The shorter, 30 minutes, pre-treatment with hypertonic mannitol did not seem to affect the shape of the curve, but the 'step' was not apparent in the time course following the longer treatment. This latter result cannot readily be explained, unless one postulates that the leaves, after this pre-treatment have a density high enough, due to greater permeation into the leaf following plasmolysis, to enable them to continue sinking through the viscous paraffin for 10 minutes. After this time the mannitol was being displaced by the paraffin, and so increase in weight continued.

Mannitol is immediately displaced by water and so no 'step' appears in this time course.

c) Determination of the volume of the A.F.S. and W.F.S.

It appeared that the A.F.S. was filled with mannitol in 38 minutes. This value was obtained from close examination of the data (Figure 10), and the fact that the final result of displacement of mannitol by paraffin, which should have given an answer of zero in the differential weighing, gave results which were slightly negative. This indicates that not all the A.F.S. had been occupied in the 30 minute mannitol treatment. After 5 minutes in paraffin the tissue, with its A.F.S. filled with mannitol, had settled before further increase in weight due to displacement of the mannitol.

The volume of the A.F.S. found by this method could be calculated as follows; for uniform tissue prepared in a standard procedure, by:

$$V \text{ of A.F.S.} = \frac{Wm - Wp}{Pp - Pm}$$

where Wm = weight in mannitol after 38 minutes
Wp = weight in paraffin after 5 minutes
Pp = density of paraffin
Pm = density of mannitol.

Results obtained using this method were checked against coarse volume determinations. In one experiment the A.F.S. volume

calculated was 0.163 mls. for 0.0965 gm. fresh tissue. The total volume displaced by this tissue was 0.25 mls, and as a rough assumption the fresh weight was taken to represent the volume of the non-free space. Thus the value obtained for the A.F.S., 0.163 mls., plus the volume of the non-free space, 0.096 mls, gave a total volume for the tissue of 0.259 mls. Thus the method for A.F.S. determinations gave an answer in the correct order of magnitude.

Unfortunately, this method did not give a direct indication of the volume of the W.F.S. (Water Free Space), in which the phase 1 ions are dissolved. However, many experiments showed that in leaves from the same plant, the volume of the air spaces/fresh weight found as indicated previously, and the volume of A.F.S./fresh weight did not vary by more than 5%. Therefore, as a prelude to measurements of ion concentrations in the W.F.S. of the leaves, experiments were conducted to find the mean % part of the A.F.S. occupied by the W.F.S., in the leaves of each experimental plant.

Hence:-

W.F.S. = A.F.S. - volume of intercellular air spaces

Figure 12

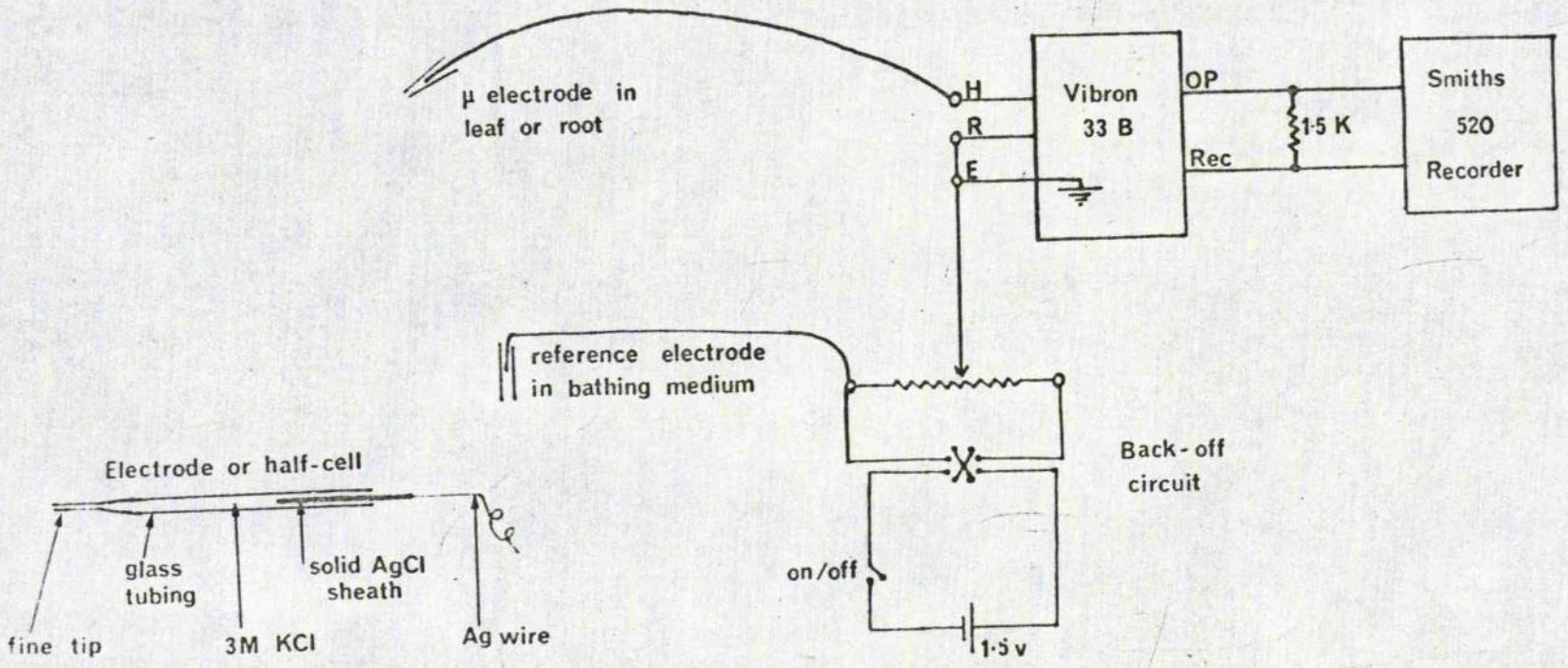
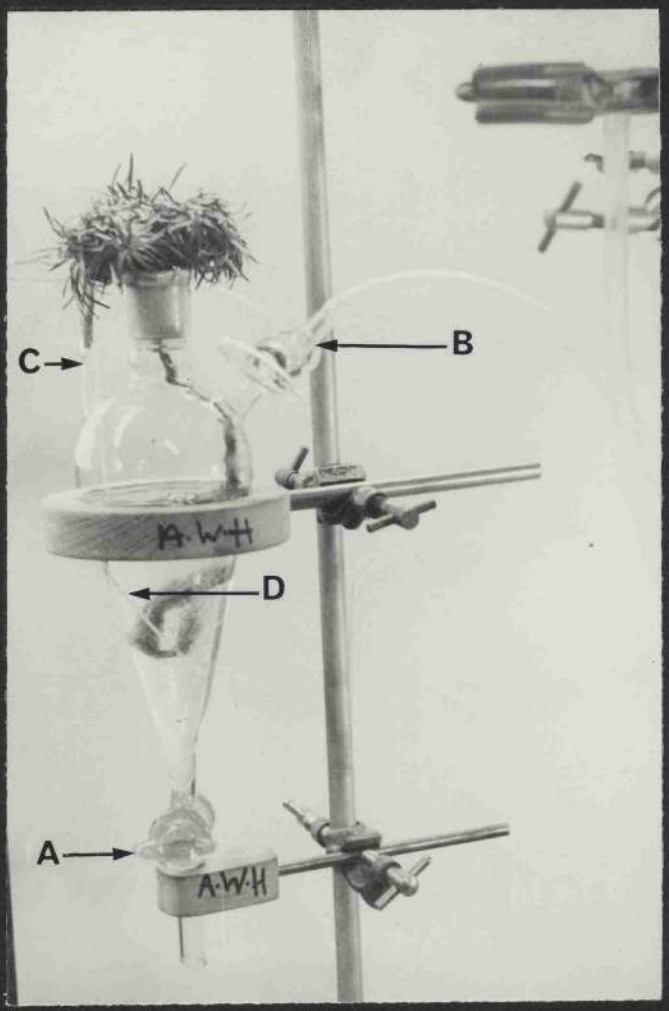

μ electrode in leaf or root
H
R
E
Vibron 33 B
OP
Rec
1·5 K
Smiths 520 Recorder
reference electrode in bathing medium
Back-off circuit
on/off
1·5 v
Electrode or half-cell
glass tubing
solid AgCl sheath
fine tip
3M KCl
Ag wire

Plate 7



Section 6.

Electrical measurements

Measurements of electrical potential between the leaf free space and medium bathing the roots, and between individual cells in the roots and the bathing medium were made. The electrodes used were silver wires coated with silver chloride, inserted into 3M. KCl salt bridges. The potential between a micro-electrode in the tissue, and a reference electrode in the bathing medium was measured on a high impedance electrometer (Vibron 33B), so that no current was drawn from the system by the electrometer. (Figure 12)

Tip junction potentials (Adrian 1956), caused by unequal rates of diffusion of K^+ and Cl^- through the narrow micro-electrode tip, were minimised in the reference electrode by use of wide-diameter (1/8") tubing filled with 3M.KCl/agar.

a) E.M.F. gradient across the whole plant.

Plants were suspended from the neck of a modified separating funnel containing the experimental solution, in which the plant roots were bathed. This in turn was housed inside a ventilated wooden box, with a 12" warm white fluorescent light providing 6.95×10^{-3} cals/sec/cm^2/mμ. light energy. This arrangement is shown in plate 7.

The bathing medium could be changed by running off the

existing solution through tap A, and replacing it through tap B. The reference electrode filled with 3M.KCl/agar was suspended through the side arm C. Air, delivered from a 'Hi-flo' electrically operated pump, bubbled from a short length of 1/16" diameter polythene tubing, (D), also suspended through side arm C. Aeriation also acted as a stirring mechanism, the rate of bubbling being changed if rapid stirring was required. Evaporation from the experimental medium was prevented by keeping the taps closed, and plugging other inlets with damp cotton wool.

Before commencing measurements, a small volume of the experimental solution was run off into a small beaker, and the needle of the 'Vibron' zeroed, by use of the backing-off circuit with both electrodes immersed in the solution, so cancelling the small tip potential of the recording electrode.

The reference electrode was then replaced, and the recording electrode also 3M.KCL/agar, positioned using a right-hand Narishige micromanipulator mounted from a clamp retort stand. The pontentials were recorded on a Smith's 'Servoscribe 2' dual channel recorder.

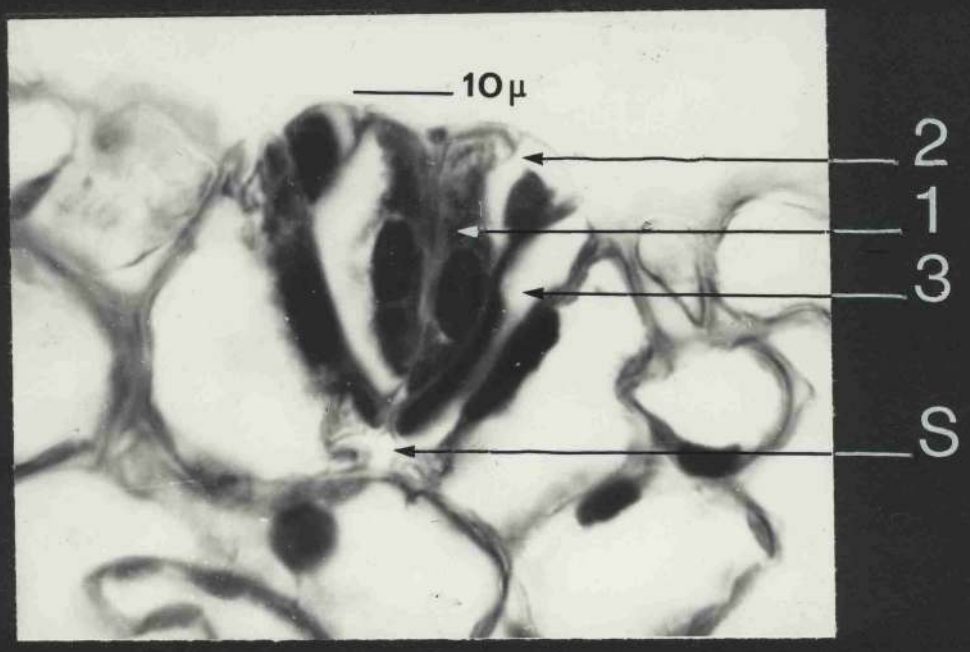
b) E.M.F. between root cells and the bathing medium

These measurements were made using 3M.KCL micro electrodes, which were pulled using a vertical traction device

Plate 1

Plate 4

A



B

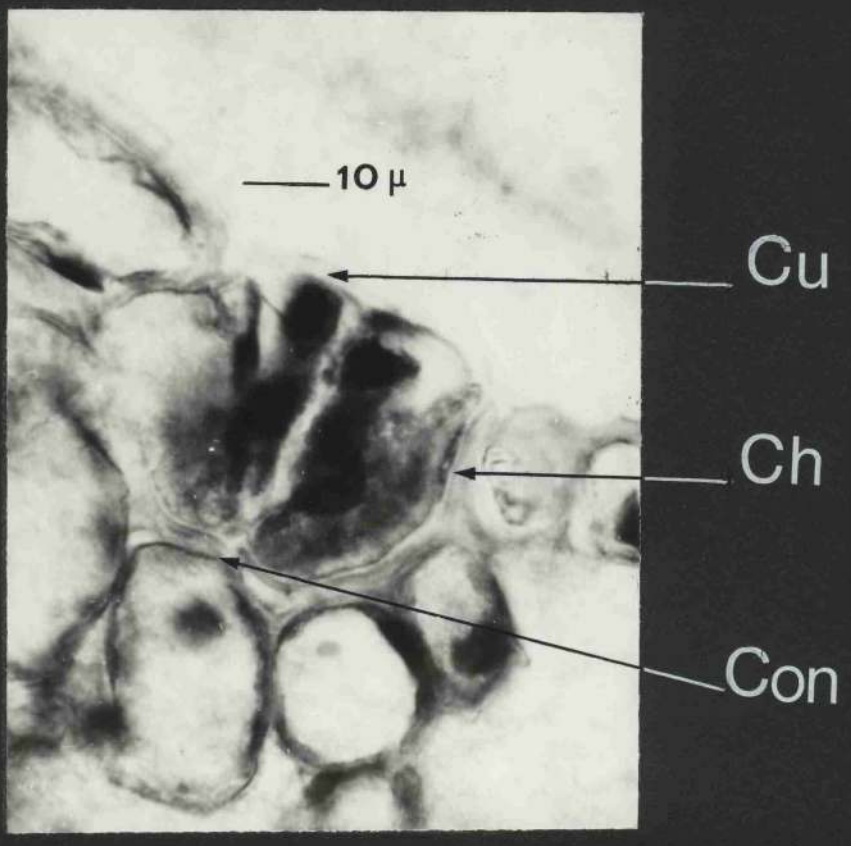
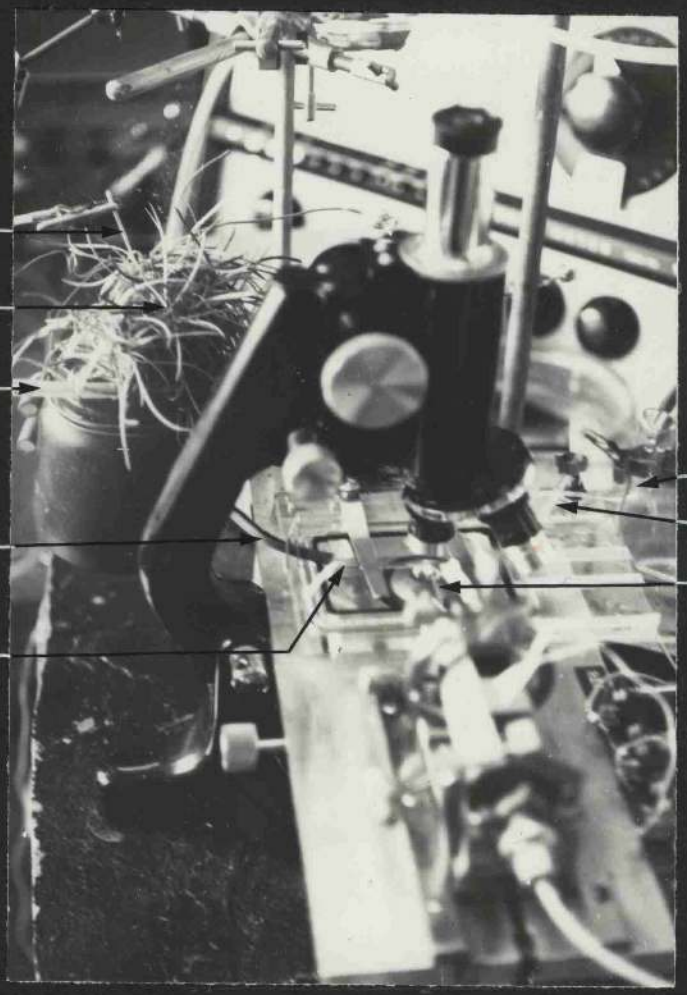


Plate 8



developed in the laboratory, the Corning Pyrex 7740 glass tubing being heated at its central region by a tungsten coil. The electrodes were filled with methanol under vacuum suction, transferred to distilled water, and finally, 48 hours before use, transferred to 3M.KCl. Initial micro-electrode tip resistances were 20-35 M-ohm which corresponded to an internal tip diameter of 0.15μm. (M.G.Stanton 1969 personal communication, by calculation from tip resistance in a defined medium, and confirmed by direct electron microscopic examination).

The apparatus for measuring these potentials is shown in plate 8. It was designed by M.G. Stanton in 1966 and built in the laboratory, and is the subject of a patents application. It allows a microelectrode to be positioned with an accuracy of within 1μm, and has no 'back-lash' or 'run-on'.

A root still attached to the experimental plant (Ex) was led along a length of polythene tubing (T) and irrigated with bathing solution by way of tubing (I), onto the adapted stage of a Watson Service 2 microscope. The root (R) was then secured in place with a coverslip held in place with stage clamps. The root under the coverslip was irrigated from the back by means of a glass pipette dropper, delivering into a narrow gauge polythene tubing via a glass funnel (F).

The vision afforded by this system is identical with that of a normal microscope.

The 3M.KCl/agar reference electrode (Ref) was positioned so that the bathing medium flowed past, as it left the vicinity of the root. Hence, there was no possibility of K and Cl ions dissolving out of the agar, and affecting the ionic concentration in the root region. The electrode for recording root potentials (Rec 1), was attached to the chuck of a micromanipulator fitted with coarse and fine adjustments in the x, y, planes, and latterly with a motorised fine advance system. A second electrode (Rec 2), is shown in the photograph, which was used in one experiment to measure the potential between leaf and root, concurrently with that between root and bathing medium.

The benefit of experimenting on a root which was attached to the plant, was that recordings such as those outlined above could be made. Also there could be no short-circuiting due to a continuous solution phase, between a microelectrode in the intercellular spaces or open-ended xylem vessels of the root, and the reference electrode.

"Backing-off" prior to use of a micro-electrode, removed the value of the tip potential from the potential measured between a cell and the bathing medium. This potential (Dainty, 1962), is the

sum of the tip potential which has been removed by backing-off; the diffusion potential across the unstirred layers on either side of the membrane; and the actual potential between the cell interior and the bathing medium. The diffusion potential is likely to be very small as the cells themselves are of small dimension, so any unstirred layer will be narrow, and the solution bathing the root flows quickly.

It was generally found that electrodes with a tip potential greater than -10mV. did not hold a steady level when backed off and so these electrodes were rejected.

Change in tip potential occurred frequently when the micro-electrode was driven across the root, due to either partial blockage of the tip with charged cellular debris, or by breakage of the tip. The occurrence of such a change was often discernible on examination of the recording, when succeeding results could be corrected for the error. If, on withdrawal of the electrode, a tip potential was obtained the source of which was not apparent on the trace, then the results obtained using this electrode were rejected.

The dependence of the tip potential on the concentration and composition of the medium in which the electrode tip is situated, lead to doubts concerning the absolute value of the electric potential recorded. (However, values for change in cell potentials with change in external concentration of the bathing medium are reliable (Adrian 1956).

Section 7.

Flux measurements on whole plants

It was necessary in the later stages of the work to know the ratio of the partial fluxes of Na and Cl, into and out of plants of *Armeria maritima* at different external concentrations. At any one time there is a net movement of ions either into, or out of the plant, unless flux equilibrium has been achieved between the plant and the bathing solution.

Direct chemical analysis will only reveal net loss or gain of any ion by the bathing medium, within the bounds of accuracy of analysis for that ion. Very small amounts of radio-activity can be detected accurately, and so use of radio-nuclides as tracers, greatly enhances the accuracy of analysis for minute changes in concentration. A known net movement of ions can be split into its components, by measuring a uni-directional movement of tracer out of the plant. This can be achieved by substituting a labelled bathing medium for an un-labelled one of the same ionic concentration, and measuring the rate of re-appearance of tracer in the bathing medium, from tissue which had previously absorbed radio-active ions. Hence, knowing the net flux, and the partial flux outwards, the partial flux inwards can be calculated, as the net flux = influx + efflux.

For the above calculation to be made, it must be assumed that although the nuclide added possesses physical properties which enable it to be analysed separately from the stable form, there is no isotopic discrimination by the plant. Hence the proportion of radioactive tracer and stable carrier initially introduced as the bathing medium, will remain constant, and loss of tracer from the solution will correspond to a proportionate loss of the carrier ion. The proportion of tracer to carrier is the specific activity, and was measured at the beginning of each experiment.

Plants were pretreated in the same media, and under the same conditions of culture as were the plants used in the experiments with which the flux measurements were to be compared.

The plants were then transferred to a vessel adapted from the lower portion of a 50 ml. burette, and housed in the ventilated chamber described in Section 6 (a). The total volume of the system was 16 mls., and the arrangement such that addition of 10 mls of experimental solution caused immersion of 2 mls. of root, having an approximate surface area of 36 $cm.^2$ Thus the ratio of surface area to volume of bathing solution was sufficient to allow measurable depletion of ions from the medium, even by ordinary analytical methods.

New solution was introduced through a side-arm to replace

solution run-off through the basal tap. The system was stirred and aerated by bubbles of air introduced through 1/16" polythene tubing connected to a 'Hi-flo' air pump.

Both isotopes were used in the form of NaCl. $^{22}NaCl$ or $Na^{36}Cl$ were added to 10 mls. of experimental medium, to give an activity of the order of 1200 counts per minute for each 50µl sample removed from the medium. The samples were taken for counting at regular intervals by removing 2 mls. of solution through the lower tap, pipetting 50µl. onto a 1" planchette, and returning the remainder of the solution through the side arm. The sample was dried and counted for radio-activity. Plant tissue was not used so self-adsorption was negligible.

Efflux measurements were made by replacing the labelled medium with un-labelled solution of equivalent composition, and successively removing all the solution, whilst replacing it with un-labelled medium at regular intervals. 50µl. samples were taken and the remainder of the sample disposed of as liquid waste.

A control experiment was performed using roots which had been killed by boiling, to determine the degree of adsorption onto the cell walls, and the glass walls of the burette.

The activity in the samples was measured on a scaler-timer counter by a solid scintillation technique, using a windowless authracene crystal fitted to a photomultiplier tube, and housed in

a Panax LC-35 lead castle. The doubly stabilised high tension supply was provided by a Panax E.H.T.6. unit.

Efficiency of counting was in the region of 60% of the calculated number of countable disintegrations per minute, and the background radiation was 1 count per minute, which was neglected in samples of high activity obtained in uptake measurements, but subtracted from counts with low activity obtained from efflux experiments.

All counting operations were performed at night when there was no variation in the mains supply, voltage and frequency. Unfortunately the counting assembly had to be connected to the single circuit supplying the workshop machinery, and so constant variations in load affected the counting rate in daytime, even though an extra voltage stabiliser unit was fitted.

Initially the time taken for 10,000 counts was recorded three times for each sample, the mean result for counts per minute being within the 95% confidence limit, but later, due to suspected occasional malfuction of the integrating circuit, the number of counts in 10 minutes was recorded. These results were reproducible and agreed to within 5% with the result obtained in timing 10,000 counts. The few suspect early results were all recounted correctly later on.

The long half lives of the two isotopes used; ^{22}Na-2.6 years, ^{36}Cl-3×10^5 years, eliminated the need for allowance to be made in the calculations for radioactive decay.

Section 5

Observations of leaf gland secretion

These were made using a Watson Stereomicroscope with X5 objective and X7 eyepiece. The 9 cm diameter glass stage was replaced by one made from perspex with a 5 cm x 0.3 cm x 0.2 cm groove cut along the diameter. This groove led at one end into a circular reservoir of 2 cm. diameter, and 0.3 cm depth, which was shelved to support a perspex 'coverslip'.

A leaf was removed from the parent plant and laid along the groove with its morphological base in the reservoir. The reservoir was then filled with experimental solution, and the leaf base re-cut under the surface of the solution. The coverslip was replaced to minimise artificial concentration of solution caused by evaporation. The leaf blade was immersed in paraffin oil so that the secretions could be observed as saline bubbles over the gland. The volume of secretion was measured using a previously-calibrated eyepiece graticule.

The leaf was illuminated by a 250w. quartz iodine lamp at a distance of 1 metre, protected by a glass water tank used as a heat filter.

Section 9.

Measurement of the energy of illumination

The energy of white illumination received by the plant leaves, from a source immediately overhead was measured using an ISCO model SRR Spectroradiometer. A 6' probe was attached, with the photo-electric cell arranged so that light would fall normally onto its surface, at the same distance from the light source as occupied by the plant leaves in each experimental condition. The photocell was covered by a single sheet of polythene when comparing the energy received by a plant in standard conditions, compared with that received by a plant enclosed in a damp polythene bag to cut down transpiration.

The total energy of illumination was calculated from an integration of the area inside the curve traced by the spectrum scanner. The irradiation was expressed in Calories/second/cm^2/nm. It was not thought necessary to distinguish between the various wavelengths in the white light spectrum used for photosynthesis.

RESULTS

CHAPTER 3.

Introduction

The root is obviously a key organ in a study of ion regulation in any plant, as it is the organ across which ions entering the plant must travel. It is likely, therefore, that the root is of prime importance in ion regulation.

A simple method of obtaining samples representative of the ionic amounts or concentrations in the cellular and extra-cellular compartments of the root, could not be found. Elution techniques which were so successful when applied to the leaves, failed because the thin roots could not be dissected to bring each component cell in direct contact with the washing medium. Thus no distinct phases could be obtained by interpreting the kinetics of elution of ions from the roots. It was not possible, therefore, to obtain all the data necessary for measurement of the electrochemical gradient for each major ion, between the different root compartments and the bathing medium. Hence the site and efficiency of permeability barriers in the root could not be estimated by a direct comparison between the electrochemical gradient for each ion, between the xylem sap and the bathing medium, and between the root compartments and an identical bathing medium.

It was hoped that measurements of electrical potentials alone between root compartments and the bathing medium, and between the leaf and the root compartments, would provide some indication of the existence of barriers to free diffusion of ions. Where movements of ions are differentially restricted across a structural barrier showing differing passive permeabilities, and selective active transport, an electrical potential gradient will be manifest across the barrier. Thus the penetration across the root by a microelectrode should result in changes in electrical potential being apparent at barriers to free ion diffusion. It was proposed, therefore, to conduct a study of the root on an electrical basis, in order to ascertain the sites of these barriers, and composition of the bathing media.

The changes in potential of the cytoplasm of the exodermis, immediately following an increase in the concentration of a series of solutions containing a single salt, were measured in the manner of Hope and Walker (1960). They were related to the relative permeability of the plasmalemma of the exodermis to the major ions. These relative permeabilities were to be used in the Goldman equation (Dainty, 1962) to predict a value for the electrical potential between xylem sap and bathing solution. Close comparison of these predicted values with those actually recorded might indicate that the permeability of the exodermis was the controlling factor in ion uptake to the xylem.

Thus an electrical study was made on the root under conditions which were to be identical with those used for the calculation of electrochemical potentials in the xylem sap of each of the major ions. It was envisaged that these purely electrical measurements could be used, in conjunction with the results of the experiment on change in electrochemical potential of ions in the xylem sap, to predict the nature and position of any root permeability barriers.

Detailed knowledge of electrical potentials between cells in the root and the bathing medium could also be used to compare predicted and actual fluxes into root compartments, should experiments using tracers yield information on the kinetics of uptake into these compartments. Radio-tracer experiments were to be performed primarily to contrast the actual partial flux ratio, into and out of the plant, with those predicted from the Ussing-Teorell equation. This data was necessary to confirm active movement of an ion against its electrochemical gradient.

Visual evidence of salt crystals being secreted at the leaf surface, presented the further possibility of an effective site of salt regulation at the leaf. The rate of secretion of salt from the glands of isolated leaves was measured with varying concentrations of ions at the leaf base. These measurements were made to reveal the extent to which the glands could control

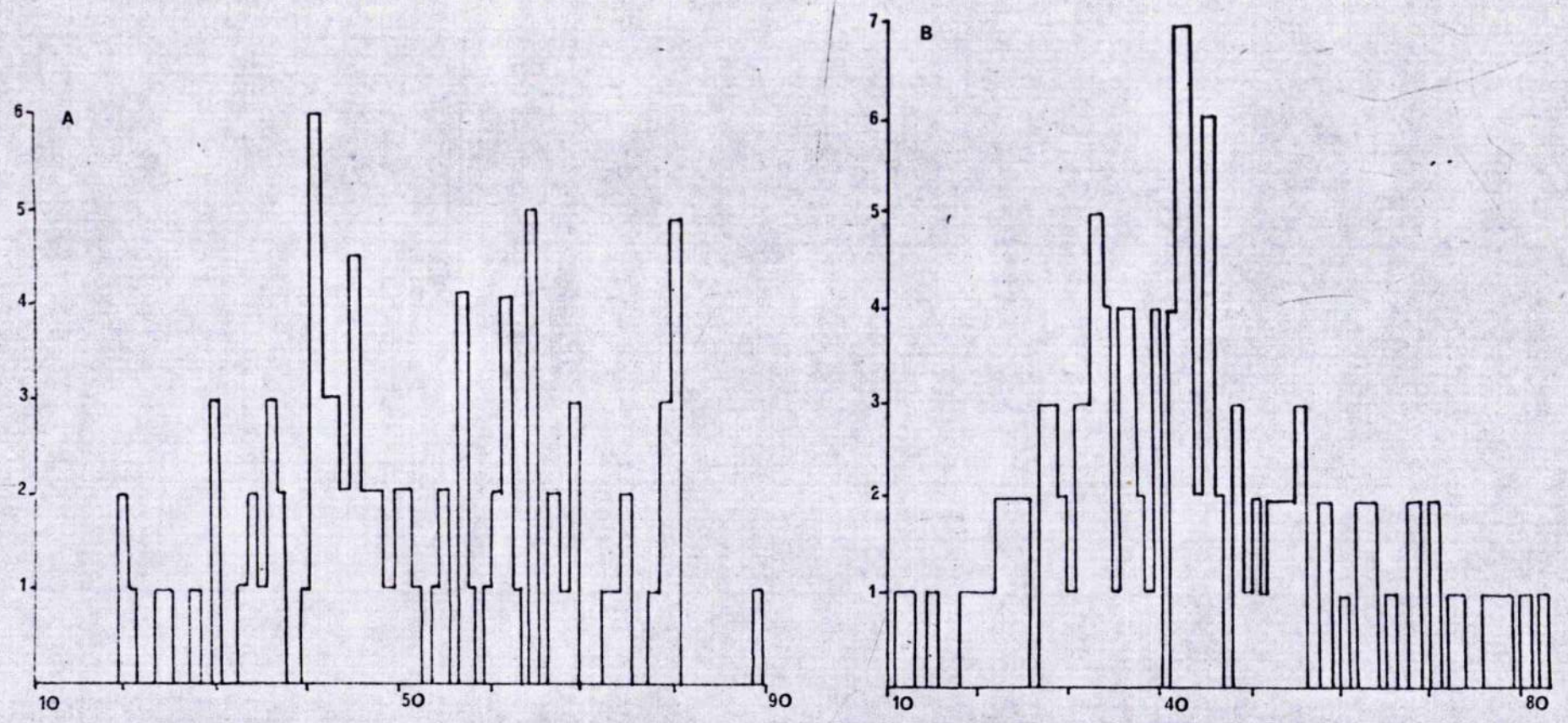
salt regulation. The actual contribution of the glands towards the maintenance of a steady level of salt concentration in the leaf, could be tested. This was done by comparing the relative changes in total salt content of the leaf, W.F.S., and the crystals washed from the leaf surface, with increase in the concentration of ions in the bathing medium.

Direct measurements of concentration of ions excreted, and the electrical potential across the gland could not be made in the time available. However, measurements of the change in ratio of the major ions relative to each other in the main leaf compartments, were made. It was proposed that these measurements would indicate preferential movement of one ion in relation to another. The conclusions could only be classified as being of an indicative nature, as they were not based on sound electrochemical principles.

Thus it was hoped that results obtained using the approaches outlined above, would lead to an understanding of the basic features of salt regulation in Armeria maritima.

Figure 13

Histograms: root exodermal potentials in normal culture



Section 1.

Measurements of electrical potential across the roots and whole plants

a) Across the root exodermis in normal culture solution

The peripheral cells of the root will henceforward be described as the exodermis. This may not always be true in the strictest anatomical sense, but it was impossible to tell, when examining a root preparation under the microscope, whether the bounding cells were of the piliferous layer or of the exodermis.

Before experiments based on a comparison of the exodermal potentials from different roots in different solutions could be started, it was necessary to know how constant were the electrical potentials recorded from a selection of roots.

The electrical potential between exodermal cells of attached roots of plants from the different races and normal culture solution were measured. The results are shown in the form of histograms in Figure 13. Histogram B shows that the number of cells possessing any one electrical potential in estuarine roots appears to occupy a normal distribution.

The mean value of this distribution is -43.4 mV. with a Standard Deviation of $\pm$ 16.1 mV. The median value is -43mV. In contrast histogram A, which shows the distribution of the exodermal potentials of montane plants has a mean of -53.1$\pm$16.6mV., with a median of -50mV. Visually it is obvious that the population

does not occupy a normal distribution, but that it is subdivided into 3 groups. Populations having limits of -20mV. and -60mV., -47mV and -78mV., and -66mV and -90mV. respectively were postulated. The Null hypothesis that the means of these populations were equal was tested using the 'Students' t-test. In each comparison the observed value for t exceeded that tabulated for a 5% probability of the limits being wrong, so the Null hypothesis was rejected. In montane roots it appeared, therefore that 3 populations of cells existed in the exodermis having means of -42.2 ± 9.8mV., -61.3 ± 9.3mV., and -75 ± 3.8mV.

The wide range of electrical potentials recorded for the exodermis of estuarine roots probably reflects the presence of many cells with root hairs, and the occurrence of exodermal cells exhibiting a range of surface areas. The potential recorded is inversely proportional to the surface area, and so the lower mean electrical potential for the exodermis of estuarine plants probably reflects the preponderance of cells having a larger surface area than those from montane roots. Very few root hairs were observed on montane roots.

It appeared therefore, that the sites of the three groups of exodermal cells in montane roots exhibiting the different electrical potentials needed to be elucidated before comparative work could begin.

b) Effect of distance from the root tip on exodermal cell membrane potential

Electrical potentials were measured between cells of the exodermis and culture medium at distances of 0.5, 1.5, 2.5 and 20 cms from the root tip. The mean results with their S.D. from 15 measurements in each region are shown below.

	Electrical Potential	
Distance	Estuarine plant	Montane plant
0.5 cms	-45^{+} 12.25 mV.	-37^{+} 13 mV.
1.5 "	-40^{+} 16.2 "	-50^{+} 11.5 mV.
2.5 "	-39^{+} 11.5 "	-55^{+} 18.3 "
20 "	-38^{+} 14.4 "	-56^{+} 17.5 "

The means of the exodermal potentials did not differ significantly for change in distance, but application of the t-test to the means of the montane cells showed 2 populations. The lower electrical potential recorded from the montane exodermal cells at the root tip differed, statistically at the 95% confidence limit from the means recorded from the other regions.

Two populations of the three shown in histogram A of Figure 13 for montane roots have been shown to have been due to spatial separation of the cells from which the recordings were taken.

c) Vacuolar and cytoplasmic potentials

The exact location of the tip of the recording electrode in the cell was not known in any of the preceeding results.

Figure 14

Trace showing electrode sealing into cytoplasm

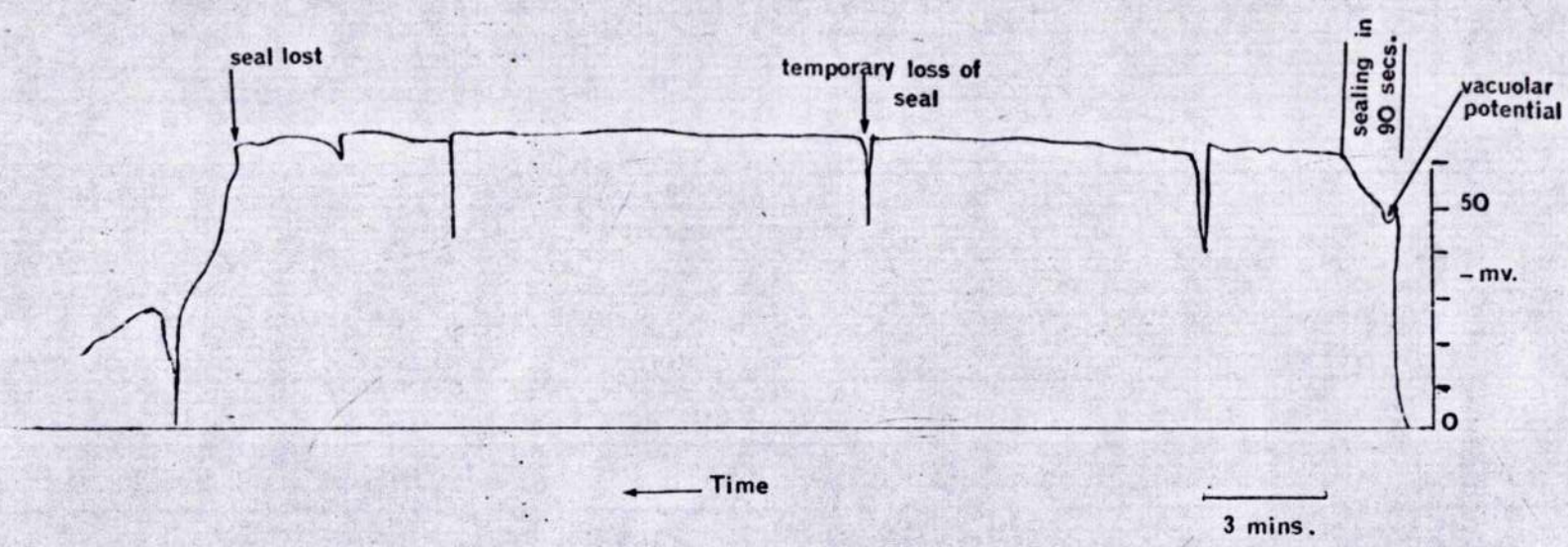
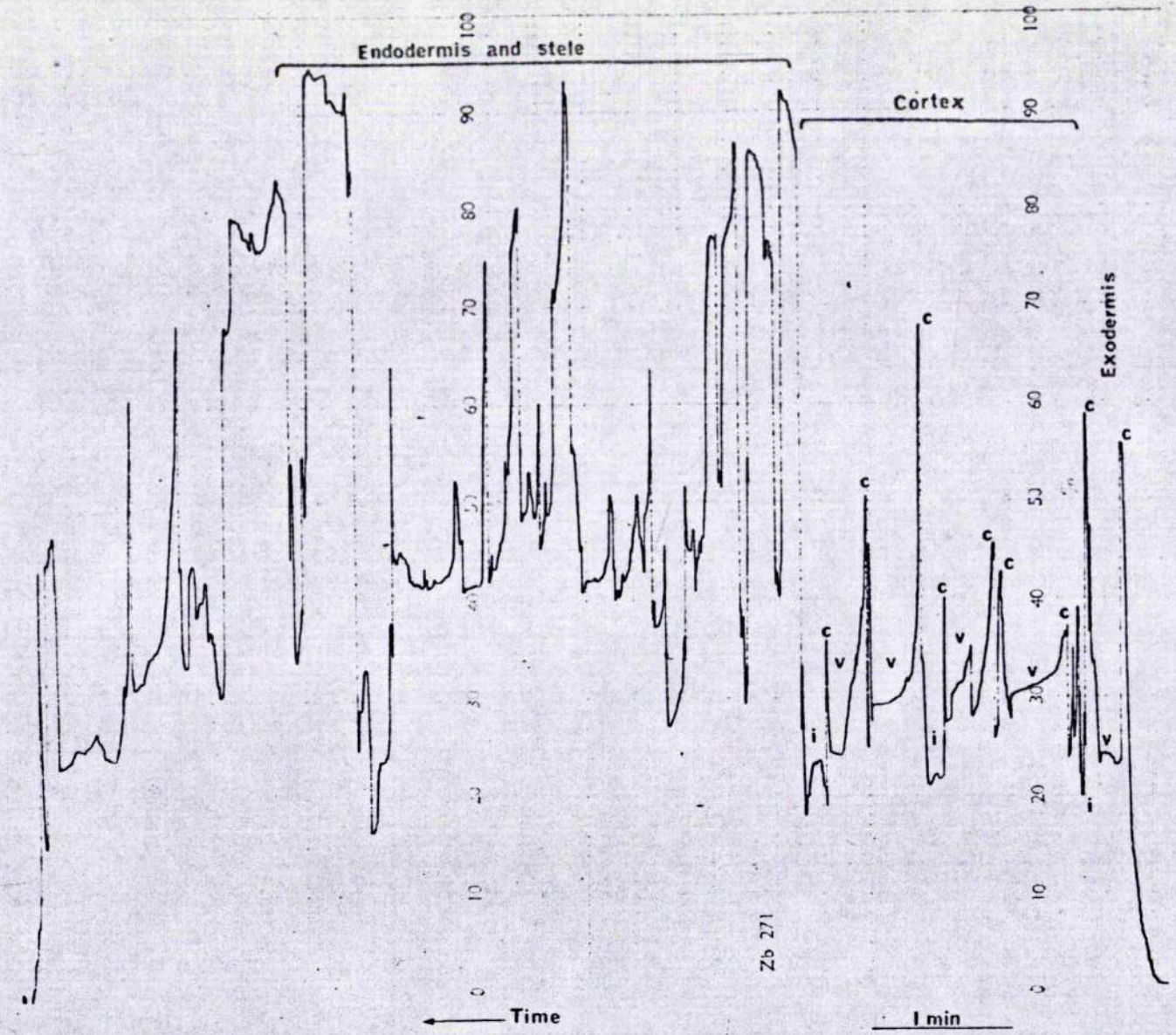


Figure 14 shows the effect of 'stabbing' the electrode into a cell and recording the change in electrical potential. Figure 14 shows such a recording. The increase in negativity after 90 seconds was attributed to the process of an electrode tip, which had been situated in the vacuole, becoming sealed into the cytoplasm in a manner similar to that described by Walker (1955). The process of sealing in is ascribed to cytoplasm flowing over an electrode tip which had been positioned just inside the tonoplast. The short breaks in the recording during which a vacuolar potential was recorded, were due to a break in the seal as cytoplasm flowed away from the tip.

Thus it can be seen that the vacuole is positive with respect to the cytoplasm, indicating perhaps that the cytoplasm contains a preponderance of immobile anions in dynamic Donnan equilibrium with the simple ionic solution in the vacuole.

All recordings considered at this stage had been made with a hand operated electrode advance. Slow advance did not cause a vacuolar potential to be recorded, but if the advance screw was jerked at the moment of penetration the electrode tip did enter the vacuole. It was considered possible that the more negative electrical potentials shown in Figure 13 A could be sub-divided on the basis of some potetials having been vacuolar in origin, and others cytoplasmic.

Figure 15



It was concluded therefore, that comparable measurements of electrical potential could be made between roots along the length of root between 2 and 10 cms. from the root tip, if care was taken to ensure that the electrode tip was located in the cytoplasm of the exodermis.

d) Electrode penetration across the root

Electrodes were driven at a rate of 48 µm/minute across the roots of estuarine and montane plants. The roots were irrigated by a steady flow of culture solution as described in Chapter 2, Section 6 (b). The region of root chosen for the experiment was between 2 and 10 cm. from the root to ensure comparability of results.

Figure 15 shows the recording trace obtained from such an experiment the recording sheet being driven at 3 cms /minute. With knowledge of the rate of movement of the recording sheet and cell dimensions it is possible to estimate the position of the electrode in the root with reference to the configuration of the trace at any particular moment. The exodermis and cortex are relatively simple to interpret; the peaks (c) corresponding to the cytoplasm, and the troughs (v) and (i) to the vacuole and intercellular space respectively. The endodermis is easy to distinguish on penetration of the stele, but on the outward journey when the electrode begins to push unsupported cells against lower structural resistance, the trace is not easy to

interpret, but does provide the expected symmetry. It is not, for example, certain which of the thick blocks on the left is the endodermis, although it is probably the first as this would leave 3 cortical and 1 exodermal cells before re-emergence of the electrode tip. This would be consistent with anatomical findings. For this reason data gleaned from trace recordings after stelar penetration was never utilised.

It will be seen that the trace was symmetrical apart from the distal exodermal cell having a smaller negative potential than that recorded from the exodermal cell penetrated initially. The tip potential of the electrode on return to the culture medium was +10 mV. This would be consistent with a breakage of the electrode at the final cell.

It would appear that a barriers to free diffusion of ions occurs at the exodermis and the endodermis. These conclusions are reached because of the presence of a negative electrical potential in the intercellular spaces, and of a high negative potential at the endodermis. The cells of the cortex show an approximately constant negative electrical potential, which is lower than that of the exodermis, and much lower than that of the endodermis. It would appear that cells of the cortex allow free interchange of ions between each other and the intercellular medium, but that free ion exchange is restricted between these cells and the exodermis and endodermis.

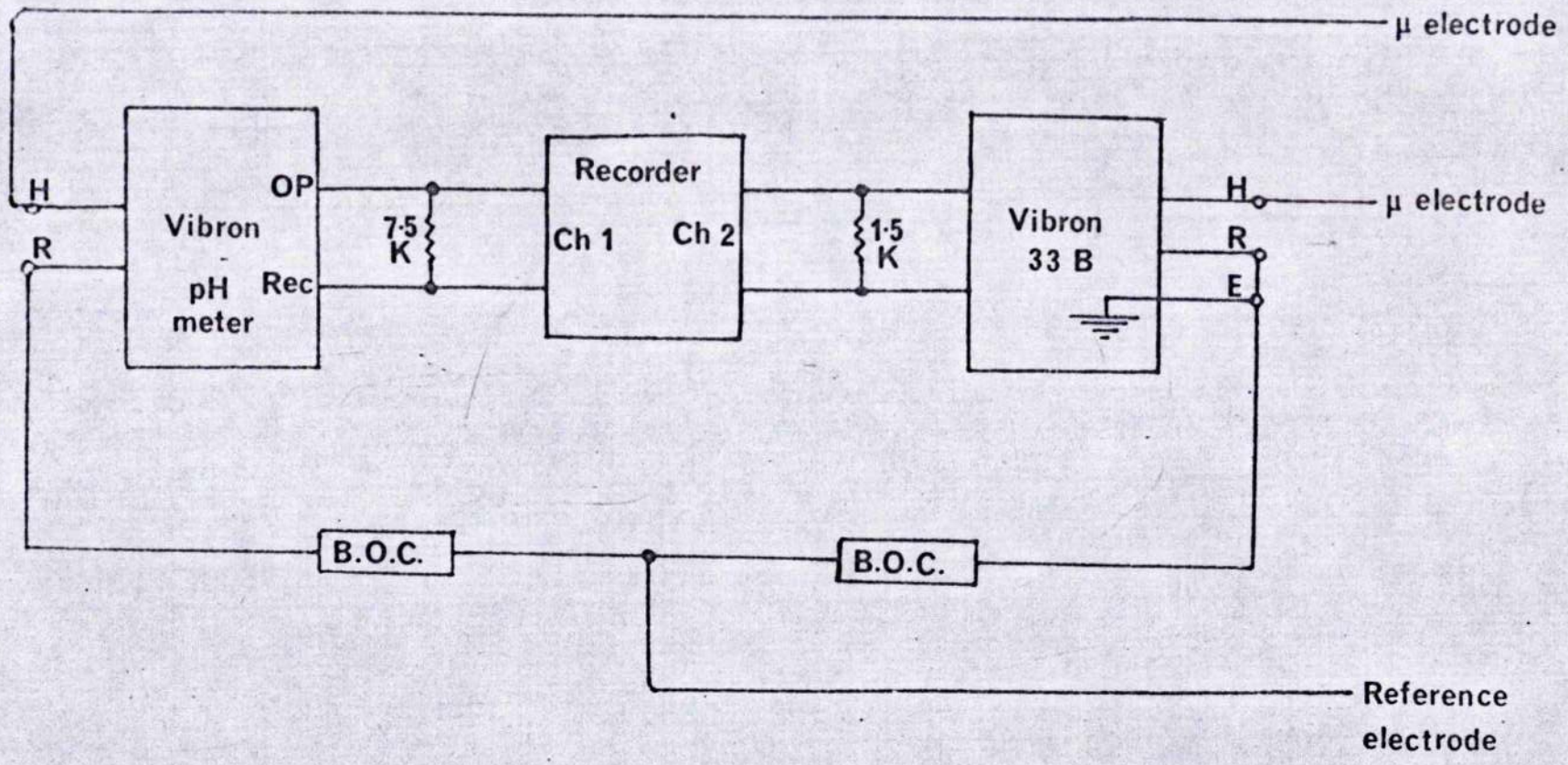
The recordings made from the stele cannot be interpreted accurately. It is possible that the large peaks were recorded from xylem vessels, as the peaks are too numerous to be associated with the small amounts of phloem found in sections. The smaller peaks may have been recorded from xylem parenchyma.

e) Simultaneous measurements of electrical potentials recorded as a microelectrode pushed through the root, and the electrical potential between the leaf and the bathing medium

It has been postulated in the preceding sub-section that both the exodermis and endodermis act as barriers to the free diffusion of ions. As a test of this hypothesis it was proposed to measure the effect of causing a temporary breach in these barriers, on the electrical potential measured between leaf and bathing medium. It was anticipated that a fall in the potential across the whole plant would occur when these barriers were breached, and that this fall would be of the order of magnitude the electrical potential across the barrier. The apparatus used is shown in Plate 8.

A μ-electrode (Rec.1) was driven across the root of an estuarine plant in the manner described previously, both to cause the breach, and to record the electrical potentials in the root compartment in the usual manner. A second electrode (Rec.2)

Figure 16


μ electrode
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Recorder
Ch 1
Ch 2
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B.O.C.
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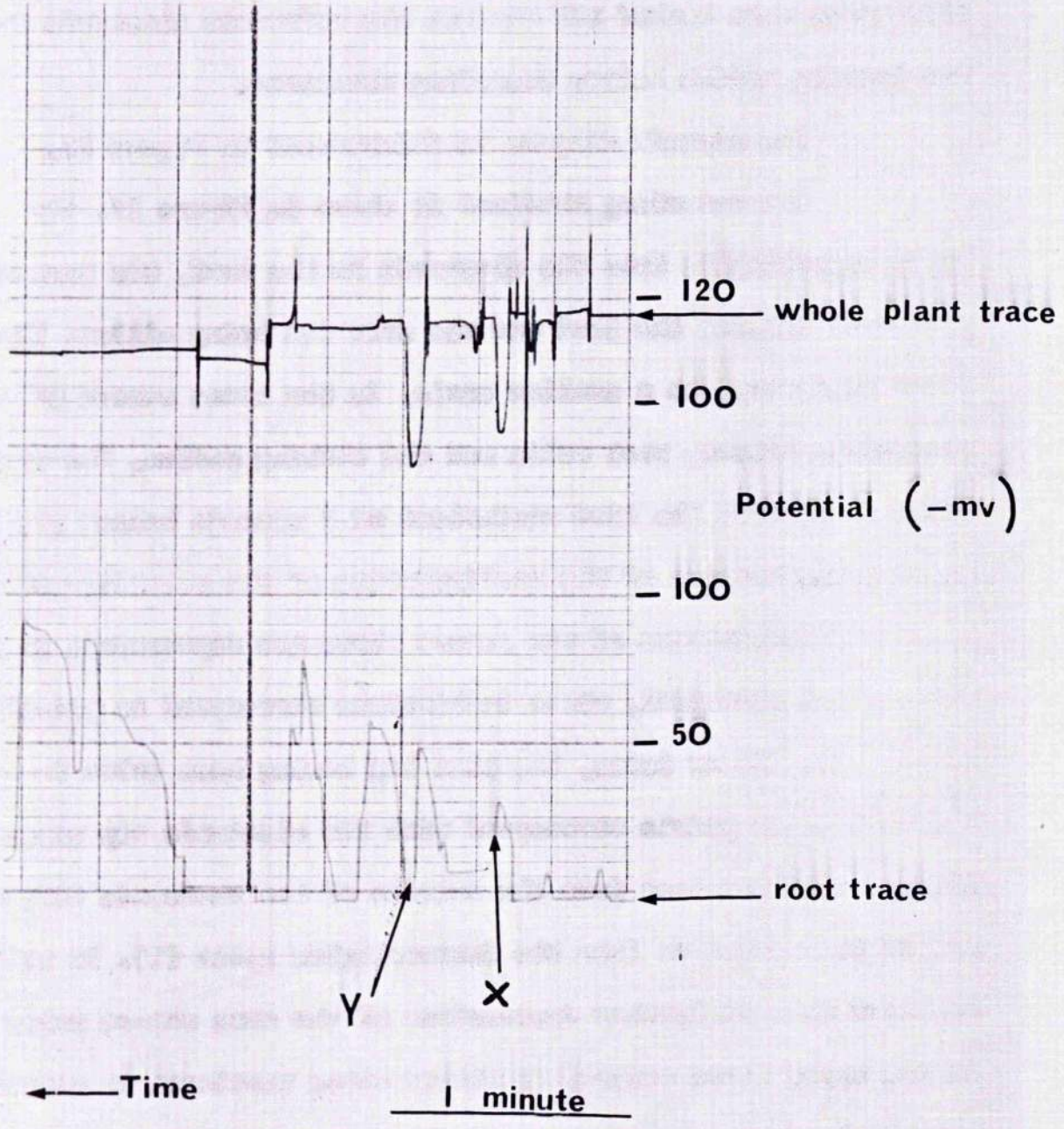
Reference
electrode

Figure 17


120
whole plant trace
100
Potential (-mv)
100
50
root trace
Y
X
Time
1 minute

was positioned in the leaf, and a common reference electrode situated in the bathing medium was used (Ref). Both recording electrodes were backed off against the reference electrode in the bathing medium before recording commenced.

The circuit diagram is illustrated in Figure 16.

The recording obtained is shown in Figure 17. The upper recording is from the electrode in the leaf, the resting potential between the leaf and the solution being -117mv. The lower recording, on a smaller scale, is the trace record of potentials between root cells and the bathing medium. The upper trace is shifted the time equivalent of 3 seconds behind the lower trace, because of the configuration of the recording device.

Examination of the record shows two depressions in the whole plant potential, whose initiations correspond to points X and Y on the lower trace, the time lag having been taken into account. These points correspond with the electrode tip passing through the cytoplasm into the vacuole of the endodermis (X), and out of the cytoplasm into the intercellular space (Y). It will be noted that no further depressions of the same nature occur in the upper trace record. The lower trace continued to record potentials from cortical cells as the electrode pushed towards the stele.

The values for the transient depression of whole plant

potential are 26 mV and 30 mV, occurring immediately after exodermal cytoplasmic peaks of -30 mV. and -45 mV.

The depressions in whole plant potential are connected with the passage of the microelectrode through the cytoplasm of the exodermis. No effect was recorded when the microelectrode penetrated subsequent cells. It was therefore, tentatively concluded on this basis that the exodermis of the root acts as a barrier between the ionic environment to the inside, and the bathing medium. This barrier was temporarily broken when the electrode tip pierced the cytoplasm, presumably allowing transient continuity of the phases inside and outside the exodermis, until the forward motion of the electrode sealed the gap produced.

Unfortunately, no record was obtained of the effect of penetration of the endodermis on the electrical potential between leaf and bathing solution in this experiment. This was due to the failure of the electrode placed in the leaf to remain in position. The permanent drop in potential of 9 mV. in the whole plant potential was caused by disturbance of the leads to the electrode in the leaf.

This experiment was extremely difficult to set up, and most recordings completed were spoiled by electrical interference. None of the leads could be earthed as this would have affected the

ability to 'back-off' each recording electrode against a common reference. Manipulation of the electrodes was an intricate process in normal circumstances, but doing so without disturbing an electrode in the leaf was almost impossible. A satisfactory recording was never obtained in the time available, of the effect of penetration of the endodermis on the electrical potential between leaf and bathing solution.

f) Comparison of electrical potentials between the leaf and the bathing solution surrounding a complete root system, with those recorded between leaf and bathing solution flowing over one intact root.

It has been seen from the preceeding sub-section that an electrical potential of the order of -120 mV. was recorded between leaf of an estuarine plant and the solution bathing one intact root. This result was comparable with several recorded during this experiment. However, measurement of the electrical potential between the leaf and medium surrounding an entire root system was -93$^{+}_{-}$ 8.2 mV. for estuarine, and -81$^{+}_{-}$ 15.2 mV. for montane plants.

Thus the readings obtained for the electrical potential across estuarine plants differed by an order of -30 mV. in the two experiments.

It was possible that contamination of the 300 ml. of culture solution bathing the entire root system occurred by

leakage of K from the reference electrode. However, in an experiment described later (Section 4 (a)), it was shown that over a two hour period of continuous recording from an electrode in the leaf, no drift in electrical potential occurred. Thus any diffusion of K from the reference electrode had no effect on the electrical potential between leaf and bathing solution.

It can be postulated that, in the complete root system there are several roots which have been damaged, so that the close fitting exodermal cells no longer form an intact barrier to free diffusion of ions into the root. In this case a lower electrical potential may be expected between leaf and bathing medium, than that recorded if the barrier were intact. The electrical potential measured across the whole plant would record that of least electrical resistance, that is, the exodermis would be short circuited. Perfect roots only were used when the electrical potential was measured between leaf and solution bathing one root. In this latter case the electrical potential would be expected to reflect the presence of the exodermis as a barrier to free ion movement into the root.

In can be seen that the fall in overall electrical potential, recorded on breach of the exodermis is of the order of -25 to -30 mV., which corresponded with the intercellular

electrical potentials measured in (d). The difference between the electrical potentials across the whole plant, measured in the different ways described above, was also of this order. It is therefore concluded, that an electrical potential of approximately -25mV. between the intercellular spaces in the cortex, and the bathing solution, is controlled by a permeability barrier at the exodermis, Breach or breakage of this barrier causes a corresponding fall in electrical potential between leaf and bathing solution.

g) Summary of Section 1

The region of the roots from which comparable electrical recordings can be made has been ascertained.

Permeability barriers controlling ion diffusion have been postulated to occur both at the exodermis and endodermis. The nature of these potentials, whether they are actively maintained or are the result of Donnan systems, or a combination of both has not been discussed.

Differences in values for the electrical potential between leaf and solution bathing a complete root system, and between leaf and bathing solution surrounding one intact root, have been shown to be compatible with the existence of a barrier to free ion diffusion at the exodermis of estuarine roots.

Section 2. Determination of the relative permeability of the exodermal plasmalemma to Na, K and Cl.

a) Introduction

Electrical evidence obtained in Chapter 3, Section 1 has shown the existence of barriers to free diffusion of ions into the stele. These were situated at both the exodermis and endodermis.

The relative permeability to each of the major ions shown by the final barrier to ion uptake to the roots, will regulate the concentration of ions in the xylem sap. Knowledge of these permeabilities together with values for the ionic concentrations in the xylem sap and the bathing solution, will enable the electrical potential between these phases to be calculated using the Goldman equation (Dainty, 1962).

$$E = \frac{RT}{F} \ln. \frac{pNa\,(Nae) + pK\,(Ko) + pCl\,(Cli)}{pNa\,(Nai) + pK\,(Ki) + pCl\,(Clo)}$$

pNa, pK, pCl refer to the permeabilities of the membranes under consideration to Na, K and Cl. (ion i), (ion o) are the respective concentrations of ions in the leaf W.F.S. (xylem sap), and the bathing medium.

Values for the actual electrical potential between the xylem sap and bathing solution, together with the concentration of major ions in these phases, were to be obtained at a later stage of the research.

Comparison between values for E predicted, on the basis of relative ion permeability at both the exodermis and endodermis, and those actually recorded, would help to elucidate the site of the control of free ion movement into the xylem.

The Goldman equation required only relative values of membrane permeability to each ion. Thus detailed measurement and comparison of actual fluxes need not be made to provide absolute values for membrane permeability to each ion. Accordingly a simple approach was envisaged.

If a single cell is pictured as being surrounded by a dilute solution of 1 fully dissociated salt, it will possess an electrical potential between its cytoplasm and the dilute solution. If the concentration of this solution is suddenly raised, the membrane potential will only remain constant should the permeability of the membrane be the same for both ion species. A change in potential in a positive direction will be caused if the cation is more permeable, and the reverse, if the anion is more permeable. Substitution of one ion of the pair for another of the same sign will cause a change in the potential recorded, which is a function of the relative permeability of the membrane to the interchanged ions.

Alternatively, comparisons of change in potential attributable to increase in concentration of 1 ion only, can be made

if the ion under consideration is accompanied by a large, indiffusible, organic counterion.

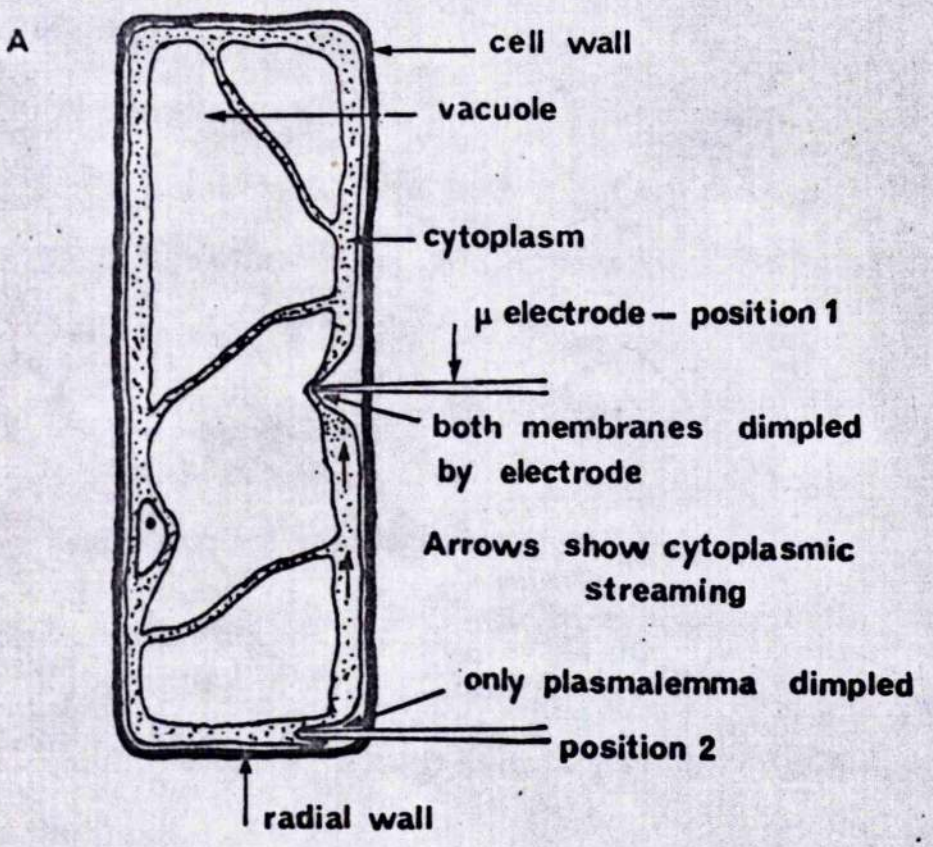
Hence values of relative permeability of the membrane to each major ion, can be studied by increasing the concentration of single, or pairs, of ions in the bathing solution over a controlled range, and by concurrent measurement of the change in electrical potential across the membrane.

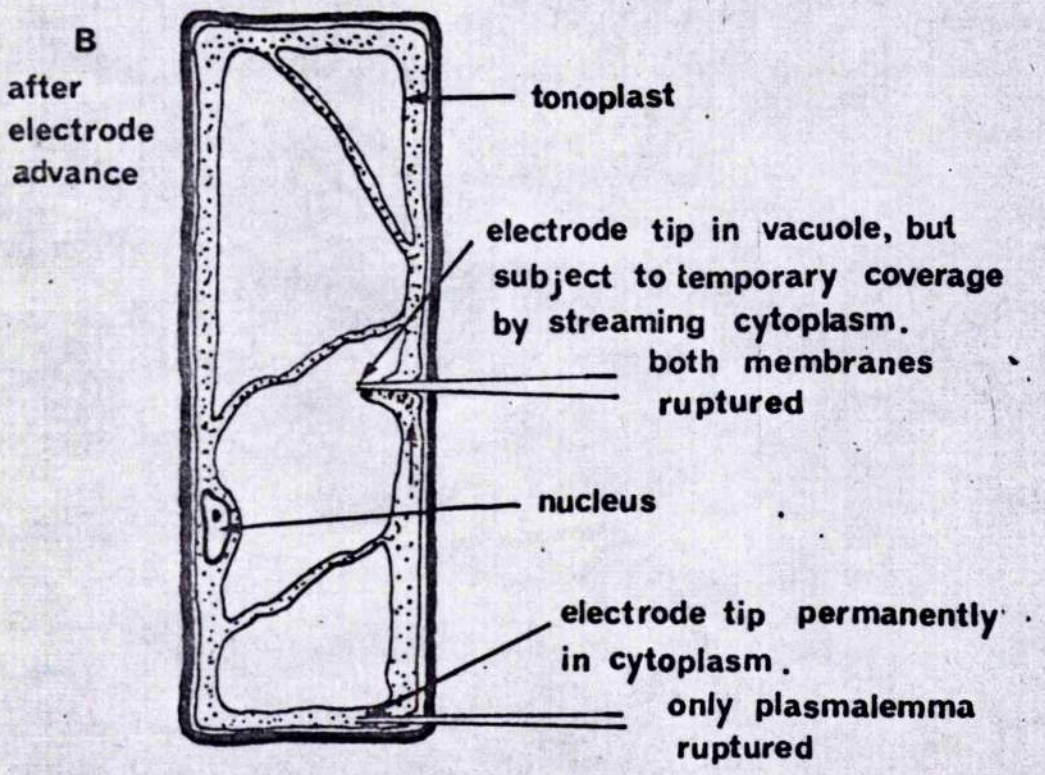
This approach, which is basically that of Hope and Walker (1960) can be applied to any cell in the exodermis of Anneria maritima, providing a recording electrode can be accurately held in position. Unfortunately the position of the endodermis, as a deeply seated barrier, precluded use of this technique because the concentration of ions could not be regulated at its surface, as it could be at the exodermis, and because an electrode could not be positioned reliably in this tissue.

Thus, time-consuming methods involving use of radio-tracers, accurate knowledge of internal ionic concentrations, absolute and partial fluxes for determination of absolute values for permeability were not utilised. Although no values for relative permeability of the endodermis could be obtained in a simple study, it was hoped that values obtained for the exodermis would be of use, as a test of the efficiency of the exodermis as a major permeability barrier in the root.

Figure 18 (1)

The effect of different μelectrode positioning on root cell penetration.





b) Manipulation of the μ-electrode

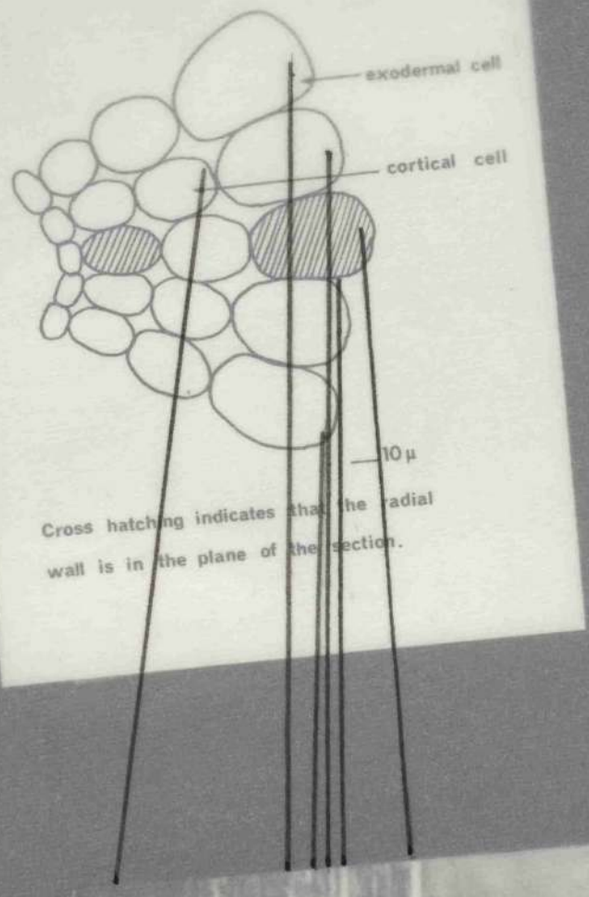
As has been stated, the essence of this experiment lay in maintaining an electrode in the cytoplasm of exodermal cell, whilst the external ionic concentration was increased. Previous experience of the effect of the 'sealing-in' of an electrode into the cytoplasm, led to rejection of this method, as a stable recording was not produced.

It was therefore decided to utilise the full capacity of micromanipulation possible, in order to place an electrode tip accurately in the cytoplasm of an exodermal cell.

Figure 18 (1) shows a diagrammatic root cell being pierced by a microelectrode in two positions. Position 1 can be located anywhere along the longitudinal axis of the cell. It can be seen that as the electrode is slowly advanced the cytoplasm 'dimples' under the pressure of the electrode tip, so that the plasmalemma is forced against the tonoplast. As the electrode is driven further, the membranes rupture simultaneously leaving the electrode tip exposed in the vacuole (diagram B). If the tip is marginally in the vacuole it will be subjected to the 'sealing-in' process as streaming cytoplasm covers the tip. The tip may, however, be too near the centre of the vacuole for this process to occur. If, however, the electrode is advanced into the cell in position 2, alongside the radial wall of the cell, only the plasmalemma is dimpled. Further advance

Plate 9

DIAGRAM: Peripheral regions of root—T.S.



Cross hatching indicates that the radial wall is in the plane of the section.

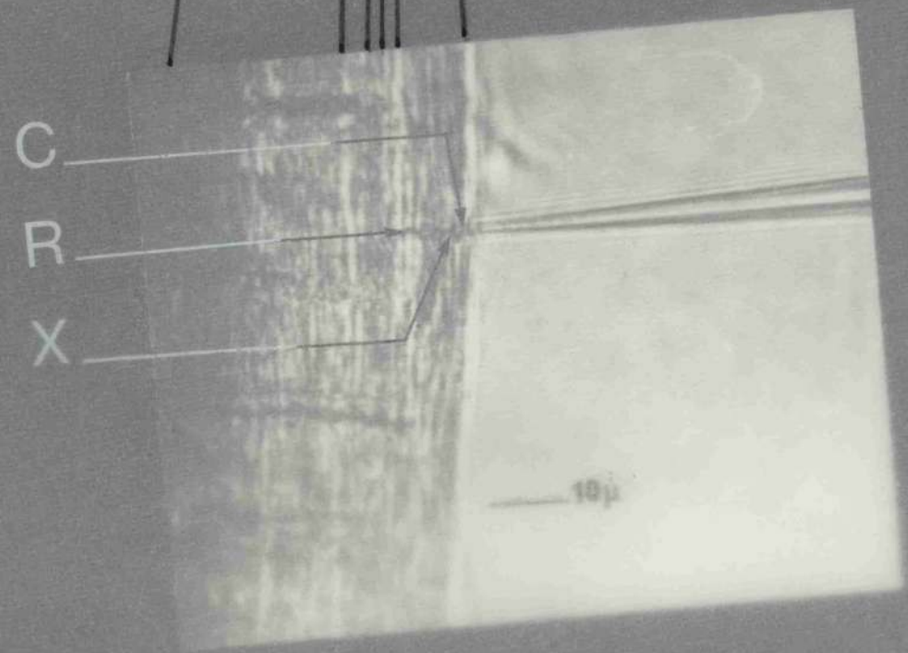
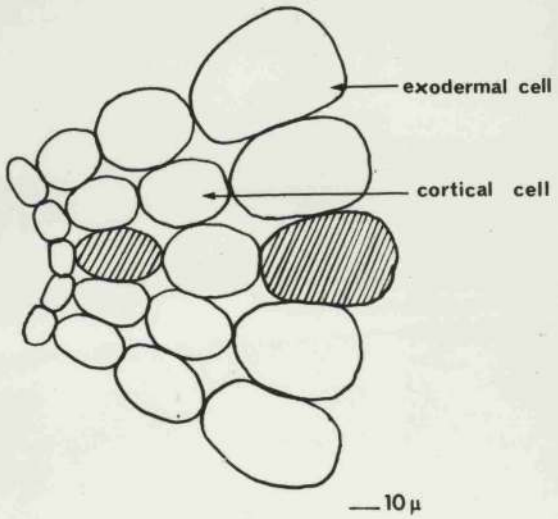
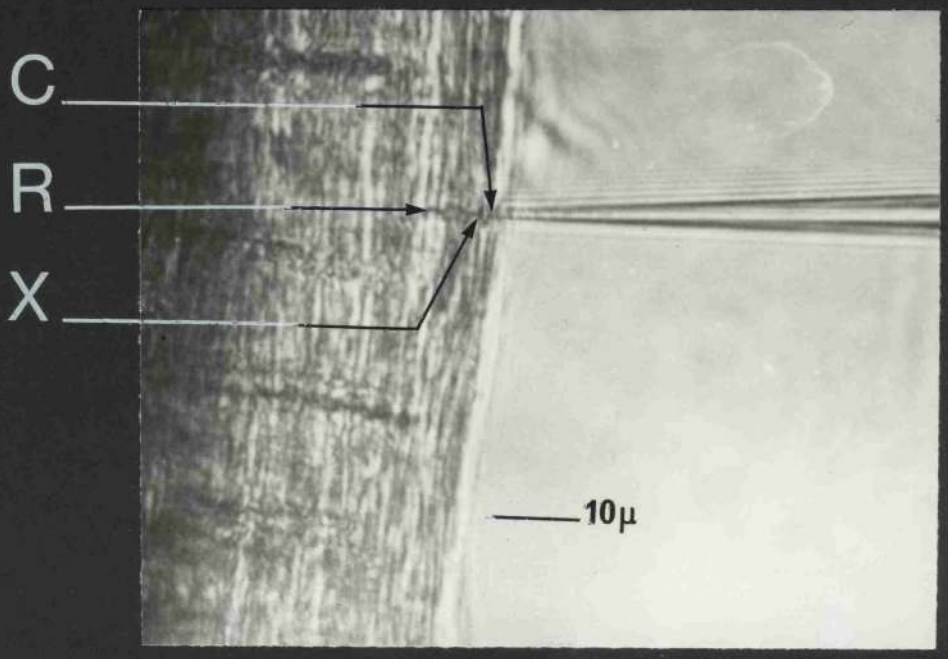


Plate 9

DIAGRAM: Peripheral regions of root–T.S.



Cross hatching indicates that the radial wall is in the plane of the section.



causes rupture of the plasmalemma, and the electrode tip is left positioned inside the cytoplasm lining the radial wall. This position can be maintained providing an adequate seal has been made at the plasmalemma/electrode interface.

Thus it should be possible to obtain cytoplasmic potential readings indefinitely, from an electrode positioned along the radial wall, unless diffusion of KCl from the electrode tip affects the potential.

The above manipulation is easy in theory, but in practice took several weeks to perfect, when applied to the exodermal cells of Amaeria maritima. The photograph of Plate 9 shows a micro-electrode in position in the cytoplasm of an exodermal cell. The electrode tip, located in the region X, appears to be under a bridge of cytoplasm C, and directly alongside a radial wall, R. The bridging effect of the cytoplasm was probably caused by vibration of the electrode tip when the shutter of the camera was released.

The scale mark on the photograph indicates that the radial wall of the cell in question is little more than 10µ in length, whereas the diagram above, based on sections cut, indicate that the radial wall is of the order of 30µm. The tracing overlay of the plate is an attempt to explain this seeming anomoly. It must be remembered that a root is roughly circular in section, so that for any clear definition of a cell

in the root to be obtained, the microscope must be focussed on the "equatorial" region of the root, where part of the corresponding endodermal cell projects above and below, adjoining cells. The peripheries of these latter cells will be seen down the microscope as longitudinally running walls; to the inside, and seemingly bounding, the endodermal cell under consideration. The lines on the tracing overlay are a diagramatic attempt to explain this and the apparent multitude of longitudinally running walls.

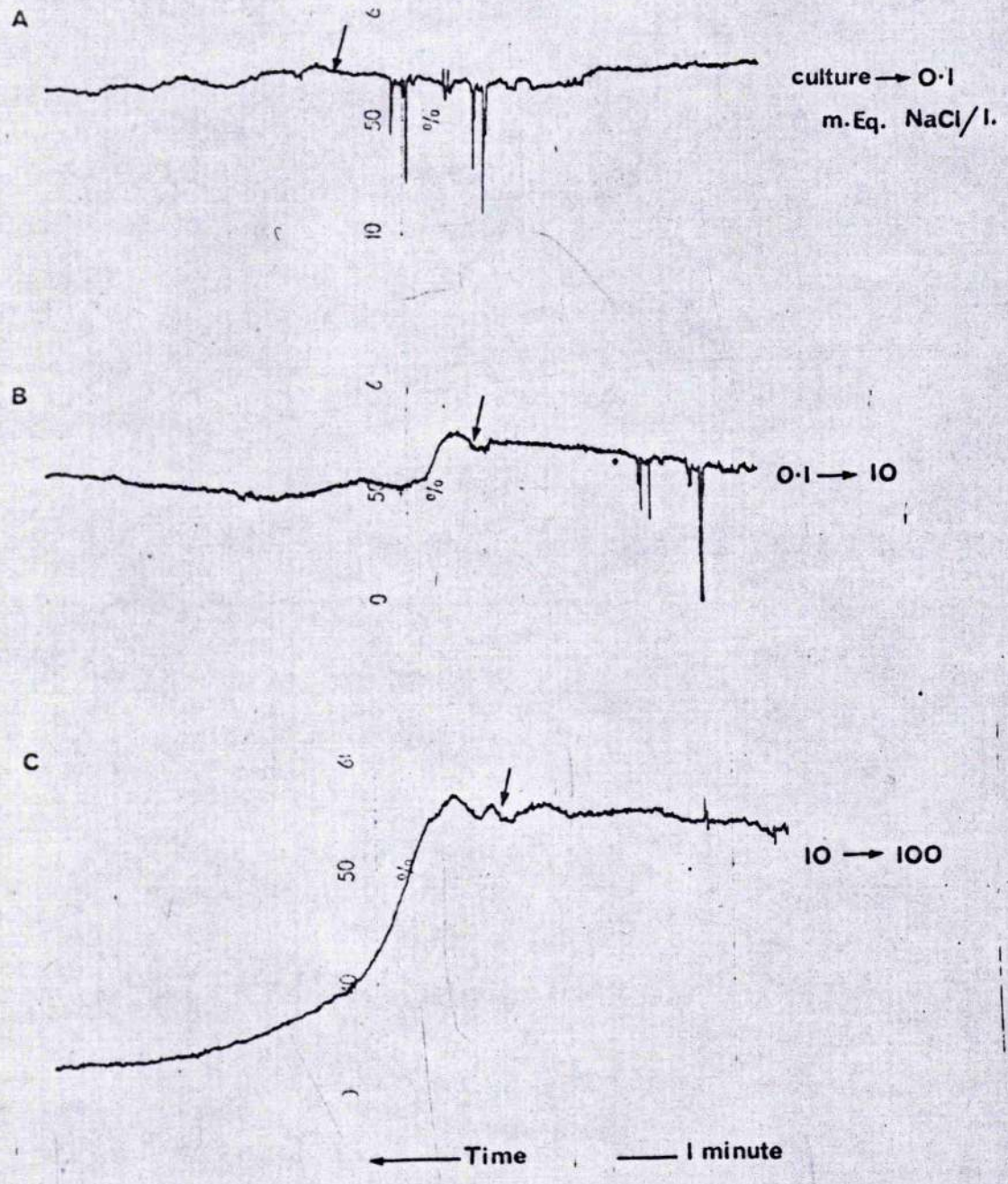
c) Solutions used

Each salt used was made up in solution in the normal dilute culture medium (Chapter 2 Section 1). The solutions were changed along a step-wise increase in concentration as indicated by the arrows below. Each experiment was conducted using a fresh root, which had not previously been exposed to medium at a higher concentration than the ordinary culture solution.

The experimental solutions were:-

Normal culture	→	normal culture	+ 1mEq/l NaCl	→ 10mEq/l	→ 100mEq/l	
"	→	"	+ 1 " /l KCl	→ 10 "	→ 100 "	
"	→	"	+ 1 " choline Cl	→ 10 "	→ 100 "	
"	→	"	+ 1 " Na citrate	→ 10 "	→ 50 "	
"	→	"	+ 1 " K citrate	10 "	→ 50 "	
"	→	"	+ 1 " Na lactate	→ 10 "	→ 50 "	
"	→	"	+ 1 " K lactate	→ 10 "	→ 50 "	

Figure 18(2)



The solutions used in the experiment were changed in a manner such that there was no interruption in flow, little change in flow rate (1.8 mls/minute), and little opportunity for mixing with the preceeding solution. The collecting funnel (F in plate 8) delivered solution to the back of the microscope slide via a short length (2") of 1/16 polythene tubing. The drip feed to this system could be cut off, and whilst the remainder of solution in the funnel drained through the tubing, a new feed from the succeeding solution was aligned over the funnel. New solution was delivered to the funnel when it was almost empty, and the drip rate adjusted. Thus contact between the solutions was restricted to the short length of tubing, and the change of concentrations at the root surface was sudden. Flow rate decreased slowly as the solution level in the funnel fell before changeover.

d) Results

Typical trace records are shown for NaCl addition to the medium surrounding an estuarine root in Figure 18 (2). The sign of the recorded electrical potential is negative. The solutions were changed when the recording had reached a steady value following an earlier change. Oscillations which can be seen on the records prior to solution change, were caused by static electricity discharging from arms reaching across the

microscope stage to complete the change.

Hope and Walker (1960) observed a change in membrane potential on increase in external ionic concentration, which could be attributed to readjustment of the Donnan equilibrium of the thick walls of Chara australis. This readjustment took less than three minutes. Any such re-equilibrium in the thin walls of Armeria maritima would be much smaller, both in magnitude and duration. In fact no evidence of Donnan re-adjustment was observed, but it could have been concealed in the slower response time of the meter and recorder circuits.

A gradual tendency towards re-attainment of the resting potential can be observed in Figure 18 (2) B. The traces are interpreted as being composed of two phases; firstly a short-term tendency towards inward diffusion, associated with membrane permeability, followed by a slow drift related to net fluxes of ions causing a change in the concentration gradient across the membrane. This study was concerned with the initial changes due to free diffusion regulated by membrane permeability, so that results were recorded as change in potential five minutes after change in solution. The total change in membrane potential caused by increase in external concentration over the range of the experiment, was taken as being the sum of the indivual five minute changes during the experiment. This 5 minute delay in

Figure 20 A

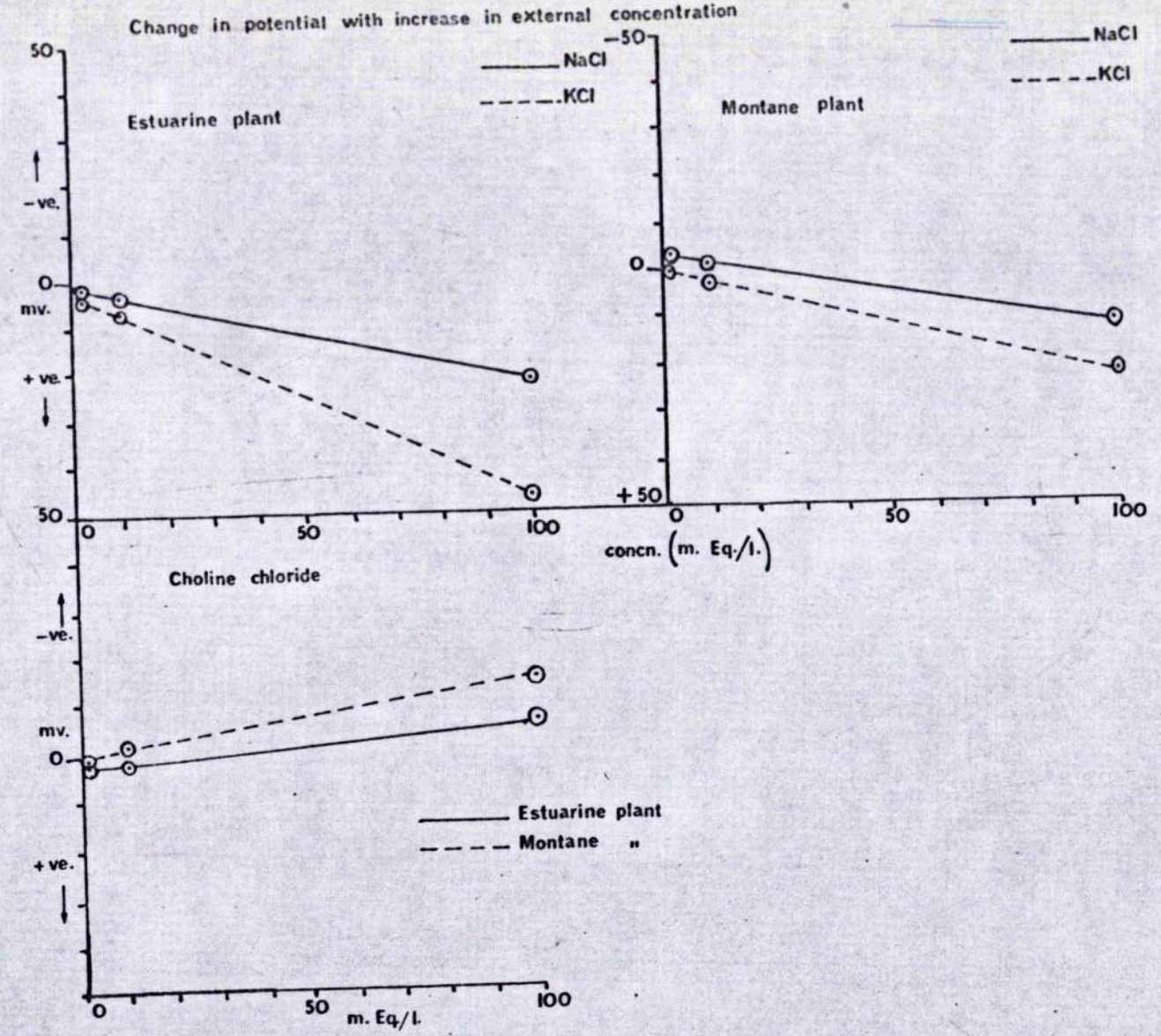
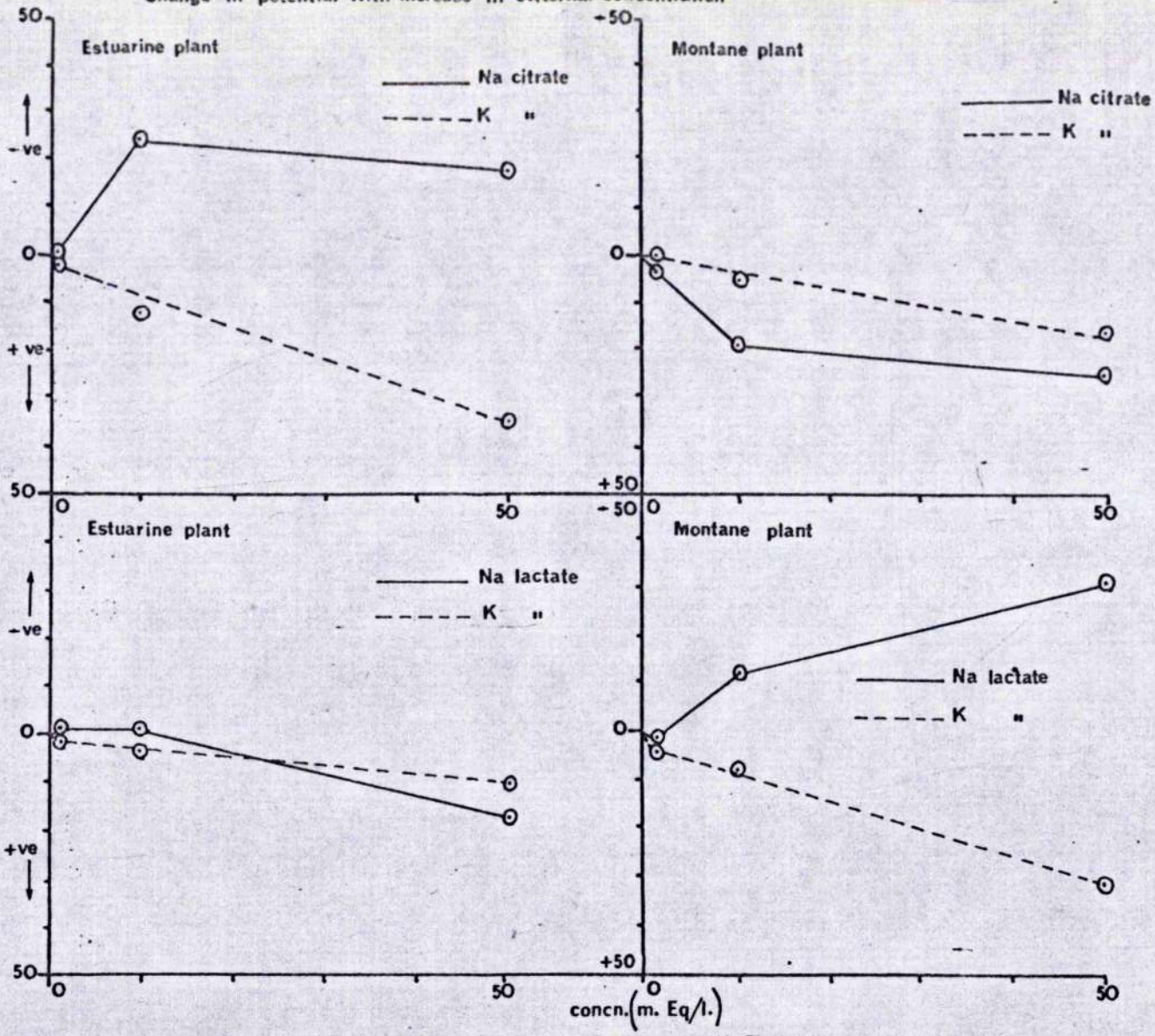

Change in potential with increase in external concentration
NaCl
KCl
Estuarine plant
Montane plant
-ve.
+ve.
mv.
50
0
-50
+50
100
concn. (m. Eq./l.)
Choline chloride
m. Eq/l.
Estuarine plant
Montane "

Figure 20 B

Change in potential with increase in external concentration



recording a value for change in electrical potential, was made to be certain the change recorded was not the result of Donnan re-equilibration.

The values for change in potential and the direction of the change, were plotted against external concentration (Figures 20A, 20B). It is noticeable that the recordings in Figure 20A are perfectly linear, indicating no change in the relative permeability of the membrane to Na, K and Cl with changing external concentrations of these ions. Each point is the mean of 2, or sometimes, values obtained from experiments on different roots.

Unfortunately, the records illustrated by Figure 20B were not so precise. In these experiments a definite change of the electrical potential of the cytoplasm was expected towards a more positive (less negative) value. This phenomenon should have been caused by the presence of an indiffusible organic anion as the counterion for Na or K. It is possible that the tendency for the membrane potential to become more negative, on increase in the concentration of the bathing medium from 1 to 10 mEq/l, was caused by loss of Ca from the cell, with the formation of insoluble Ca complexes in the cell wall and bathing medium. Thus the change in membrane potential observed was not attributable solely to controlled experimental conditions.

e) Interpretation of results

The slopes of the graphs were measured, and are tabulated below, together with the sign of change in membrane potential. Non-linear graphs were recorded as having a slope determined by the change in electrical potential across the exodermal plasmalemma, following solution changes at values of high concentration. This ensured some degree of uniformity in the interpretation.

These results are set out in Table 3.

Table 3.

Solution	Slope (mV change/mEq increase in Co)	
	Estuarine	Montane
NaCl	+ 0.2	+0.16
KCl	+ 0.43	+ 0.23
Choline Cl	- 0.09	- 0.15
Na citrate	+ 0.175	+ 0.15
K citrate	+ 0.55	+ 0.32
Na lactate	+ 0.42	- 0.47
K lactate	+ 0.16	+ 0.6

The relative permeabilities of the membranes to Na and K were calculated by dividing the slope values for KCl by those for NaCl, with the assumption of equal Cl contribution.

Thus for estuarine roots, $Na:K = \frac{\text{slope KCl}}{\text{slope NaCl}} = \frac{0.43}{0.2} = 1:2.15$

and for montane roots Na:K = 1:1.43.

These records were obtained from different roots and so as a check on the validity of comparison, the values for slope attributable to NaCl and KCl were divided by the values obtained for Choline Cl from more, different, roots.

$$\frac{NaCl}{ChoCl} = \frac{0.2}{0.09} = 2.22 \qquad \frac{KCl}{ChoCl} = \frac{0.43}{0.09} = 4.81$$

$$Na : K = 2.22:4.81 = 1:2.17.$$

Similar calculation gave a value for Na:K of 1:1.44 for montane exodermal cells. These results are surprizingly comparable.

In order to obtain the relative changes in potential for Na and Cl, it was necessary to utilise values for the change in potential with a cation present in association with an indiffusible anion. By mainly subjective assessment of results, it was decided to use the values of slopes recorded for K citrate.

These results fell on reasonably straight lines, and the value of K (estuarine) : K (Montane) of 1:0.38 compared reasonably with the K(es): K(mon) value of 1:0.66 obtained with KCl.

Accordingly, the ratio of change in potential due to K and Cl was found by:

slope of K citrate - assumed to be solely due to K = 0.35
slope of ChoCl - assumed to be solely due to Cl = 0.09

Are there any data for influx + efflux on same or similar root which (even though not time-complete etc) allow ~~calc~~ estimⁿ of flux rates from initial values thence of using

$$\begin{cases} M_{oi} = \pm P_j \cdot \frac{RT}{2F} \ln \frac{P_j J_{o+} - \dots}{P_j J_{i+} - \dots} \\ M_{io} = \pm P_j \cdot \dots \end{cases}$$

to estimate true P_j's ?

from positive directions only.

+ allow for ⊥ flux component in active dirn.

the relative changes in potential, K:Cl = 1:$\frac{0.09}{0.55}$ = 1:0.163

for estuarine roots, and similarly for montane roots; 1:0.468.

The relative effects of Na : K have already been calculated, so for estuarine roots Na:Cl = 1: (0.163 x 2.15) = 1 : 0.35, and for montane roots Na:Cl = 1:0.66.

From these results, the Cl(es) : Cl (mon) ratio is 1 : 1.87, which is in reasonable agreement with the value of 1 : 2.14 calculated on the basis of choline Cl alone.

Thus the relative permeabilities of the exodermal cells would appear to be of the order of:

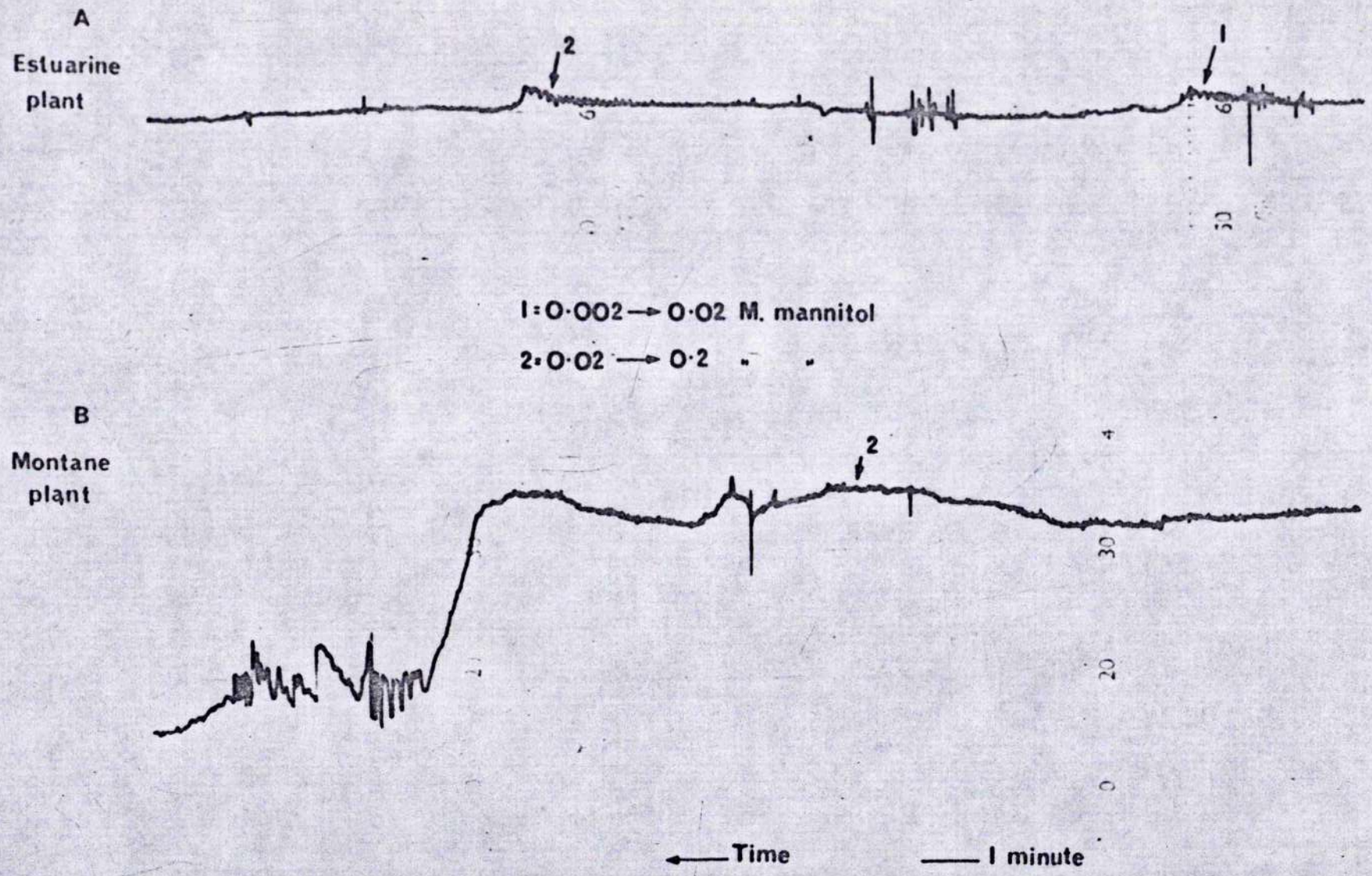
	Na	K	Cl
estuarine	1	2.15	0.35
montane	1	1.43	0.66

f) Effect of increasing the osmotic potential of the bathing solution

It was noticed during the course of the previous experiment, that following increase in external concentration from 10 - 100 mEq/l., a second drop in electrical potential recorded from the exodermis occurred. This fall was subsequent to the initial drop caused by solution change. The position of this second fall in relation, the initial drop in potential was never constant, but a recovery to its original value was recorded when the concentrated solution was replaced by normal culture medium.

Figure 21

Mannitol traces



1 = 0·002 → 0·02 M. mannitol

2 = 0·02 → 0·2 " "

It was thought unlikely that this second fall was associated with the breakdown of a regulation mechanism peculiar to montane roots, but that it was probably caused by cell plasmolysis. This latter view was reinforced on one occasion when the cytoplasm was observed to shrink away from the electrode tip.

As a test of this possibility, and also to see the effect on the cytoplasmic potential of prolonged exposure to KCl diffusing out of the electrode tip, an experiment was conducted using solutions of mannitol at concentrations which were the equivalent of the concentrated salt solutions. The effect on the potential of both estuarine and montane root cell membranes was recorded. The resultant traces are shown in Figure 21.

The upper trace(A) shows the behaviour of the resting potential of an estuarine exodermal cell, through 2 changes of mannitol, the total range being 0.002 - 0.2M. The trace maintained a constant value for the electrical potential recorded across the plasmalemma. Hence it was concluded that gain of KCl by the cytoplasm, following diffusion from the electrode tip, did not affect the membrane potential over the period of the experiment, 90 minutes. The presence of a bathing solution, exerting an osmotic potential of 4.48 atmospheres at its most

concentrated level, did not affect the membrane potential either. So it was concluded that recordings previously made, of the change in electrical potential across the membrane, were an accurate indication of degree of change in potential caused by introduction of a salt to the bathing medium.

In contrast, trace B recorded from a montane root, showed a fall in the membrane potential some 6 minutes after increase in the external concentration to 0.2M mannitol. This fall was a delayed response to increase in concentration, and so can be separated from the almost immediate response in the external concentration of a salt solution.

This latter result is condusive with the proposed explanation, that increase in the osmotic potential of the bathing medium to 4.48 atmospheres, caused plasmolysis of the exodermal protoplast in montane roots. The electrode, in effect fell out of the cytoplasm as the protoplast decreased in volume. Dilution of this medium by replacing the salt solution with normal culture solution caused turgor to be re-gained, and the electrode to be re-positioned in the cytoplasm.

g) Discussion

It would seem from the relative permeability values obtained, that the roots of montane plants are less selective than their estuarine counterparts, on the basis of the much

smaller range of permeability values. It is noticeable that estuarine roots are much more permeable to K than to Na or Cl. The variability in response to solutions containing citrate or lactate, led to a more subjective approach in the estimation of the permeability of the membranes to Cl relative to Na, but it is thought that the results finally obtained are of the right order, and taken as a whole might reflect on the difference of the normal environment of the ecotypes.

The increase in concentration of mannitol in the bathing medium did not affect the electrical equilibrium between root and solution, but did cause plasmolysis in the montane root. Unfortunately the experiment was not continued with the electrode in position, in an effort to see whether turgor was regained.

It would appear, therefore, that the protoplast of estuarine Armeria maritima after growth in the culture medium, had an osmotic potential which was greater than 4.48 atmospheres, whereas the montane plants had a marginally lower value. This again could be taken as a reflection on the plant's normal environment; the estuarine plants in relatively concentrated medium having to withstand a mugh higher external osmotic potential than the montane.

It is quite amazing that root systems which had grown in identical culture media for 18 months should show

differences, which are likely to be more marked in their natural environment. Hence, the ecotypes seem to differ in both their ratios of sap to external concentrations, and in their osmotic potentials. These differences could be maintained by intensive active transport inwards, or by slower leakage from estuarine roots, or both.

Shortage of time precluded an extensive study of the relative values of the osmotic potential of the exodermal protoplasts, but the technique of using an electrode implanted just inside the plasmalemma could provide a slow, but useful, accurate method of measurement of the osmotic potential of a protoplast.

Section 3. The effect of increase in concentrations, and change in ratio of the major ions, on epidermal and cortical cell potentials.

a) Introduction

It has been shown previously in this chapter (Section 1, (d)) that barriers to free diffusion of ions occur at the exodermis and the endodermis of the root. These conclusions were reached on the basis of records of change in electrical potential recorded as a micro-electrode passed through the root tissues. However, no discussion was advanced as to whether these electrical potential differences were actively maintained by the cells of the tissues concerned, or were the result of differential ion mobilities across cell walls, which are cation selective. If the cell walls were cation selective, a normal negative Donnan potential would be expected, whose magnitude should decrease greatly, on changing the bathing medium from a dilute culture solution, to concentrated saline. It was expected that a series of concentration increases would show the relative efficiencies of the proposed barriers, over the concentration range.

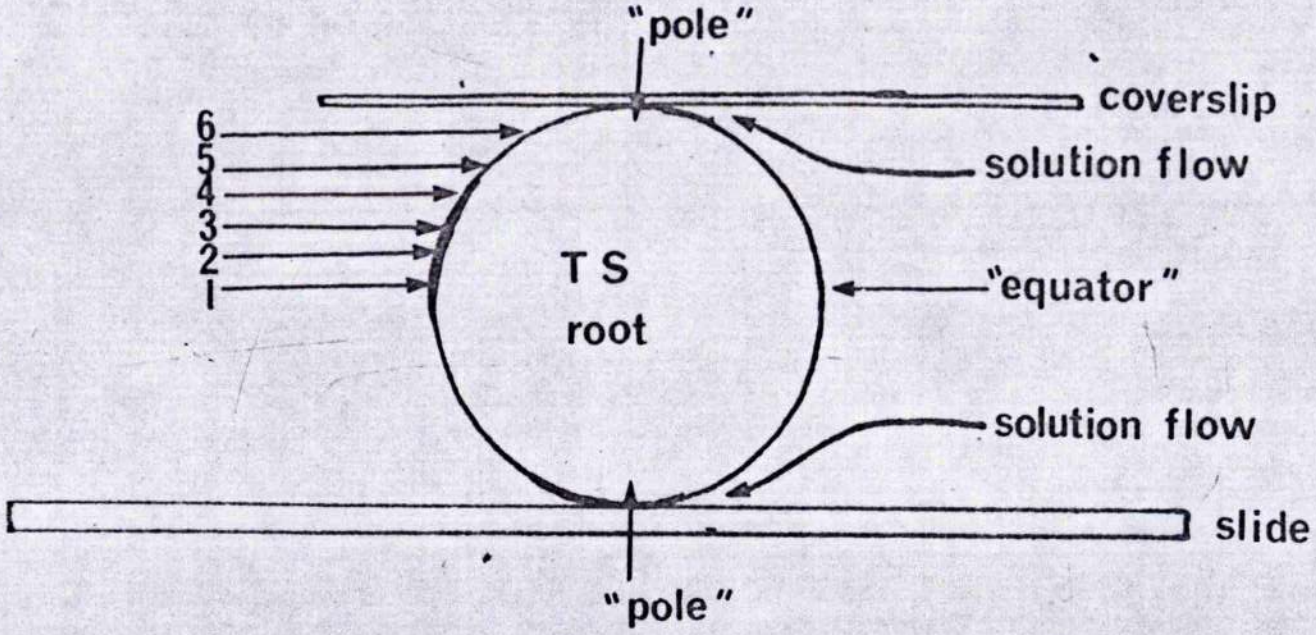
The plasmolytic effects caused by increasing the concentration of the bathing medium to more than 0.2M, precluded study of the relative permeability of the exodermis to the major ions. Hence, no values for the relative permeability

of this supposed barrier to the major ions at the concentration of artificial sea water were obtained. It was proposed, therefore, to measure the final values for the electrical potential between the exodermis and the bathing saline, over a range of Na:K ratios. If the values for relative permeability, which have been obtained in Section 2, were maintained at higher concentrations, then it would be expected that the electrical potential recorded at high K : low Na would be less negative than those recorded at high Na:low K. These deductions were based on the grounds that at a constant overall external concentration, the greater permeability of the membrane to K than Na, would cause an electrical potential to be recorded, which was lower when the medium contained a higher proportion of K than Na, than when the relative proportions were reversed. By similar argument, it would be expected that this difference would, in recorded electrical potential, would be greater in estuarine than in montane roots, as the value for K permeability relative to Na is less in montane, than in estuarine roots.

b) Method

It was hoped to obtain values for the electrical potential between the exodermis and the bathing solution, and between the cortex and bathing solution, as the concentration of the bathing medium increased. The effect of change in Na:K ratio at high concentration would also be measured. This approach

Figure 22


"pole"
coverslip
6
5
4
3
2
1
solution flow
T S
root
"equator"
solution flow
slide
"pole"

necessitated repeated penetration of recording micro-electrodes across the root. However, for a comparison of results to be attempted on a statistical basis, it was desirable to reduce the range of electrical potentials whose mean values were to be compared.

1) Position of electrode

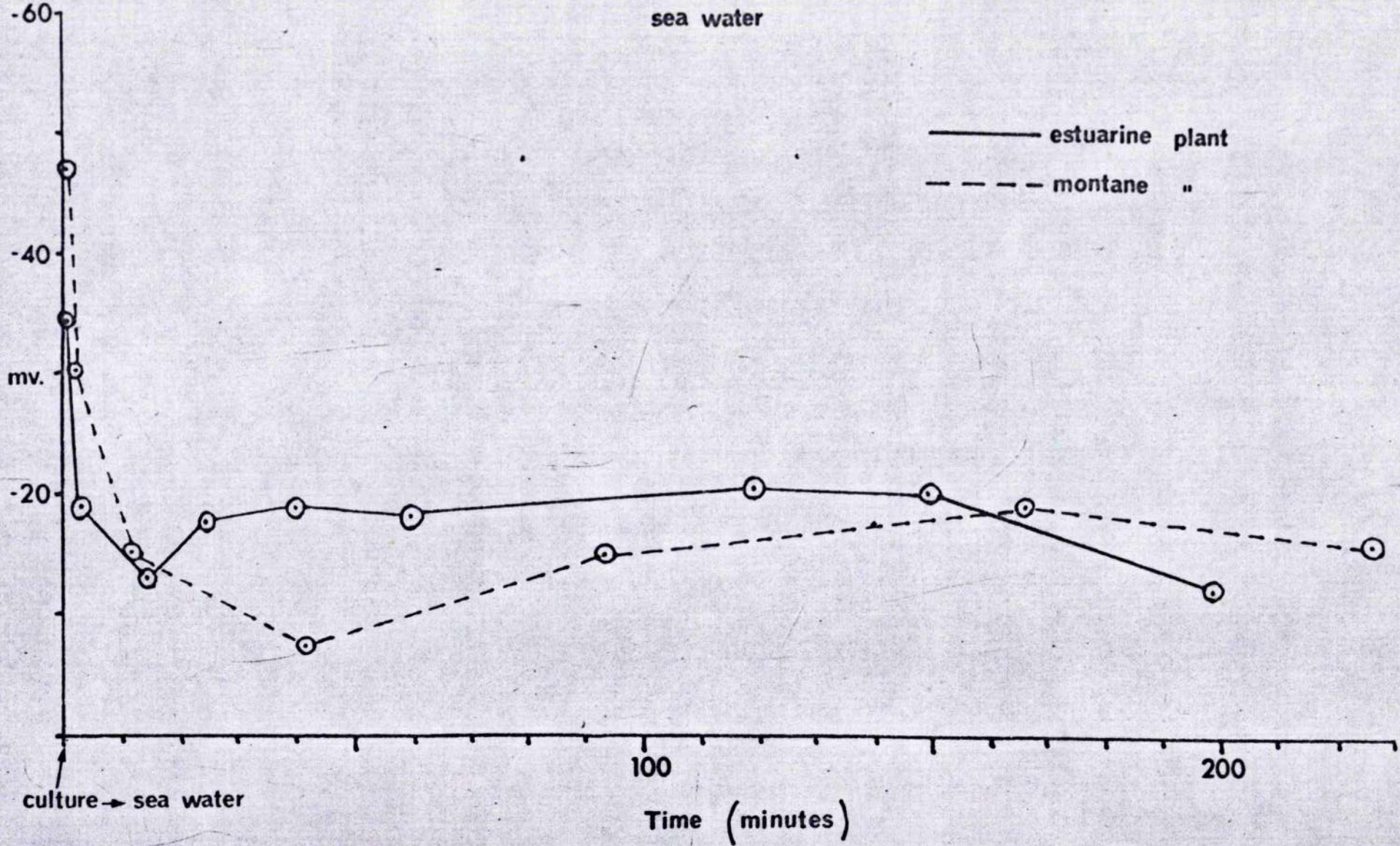
Figure 22 is a diagram of a cross section of a root, showing the direction of flow of bathing solution over its surface, when mounted in the apparatus shown in Plate 9. The numbers 1-6 refer to possible paths along which a micro-electrode may penetrate.

For measurements described in Section 2, a micro-electrode was manoevered along 1, so that it penetrated a cell at the root 'equator', allowing its position to be checked visually. However, it was found that values for electrical potentials recorded in this region varied considerably. Thus variation between readings from adjacent cells diminished as recordings were taken at positions 4,5 and 6.

The lack of consistent recordings at the 'equator' was attributed to the mass of root hairs found in this region. It was postulated that the direction of solution flow above and below the root was such that the root hairs tended to lie in the equatorial region.

Figure 23

Exodermal potentials with increase in time after introduction of artificial sea water



ii) Change in electrical potential recorded from the exodermis with increase in time after introduction of artificial sea water

Recordings were taken of the electrical potential between several exodermal cells in the regions 4-6 (Figure 22), at time intervals after substitution of culture solution for artificial sea water. The results are shown in Figure 23.

It would appear that this time course is very similar to the trace recorded in Figure 18 (2) C. In this situation the time course was monitored continuously from a single cell.

Thus, variation between exodermal cells in the region 4-6 was slight, and furthermore, constant values of exodermal potential were recorded between 10 and 150 minutes after solution change.

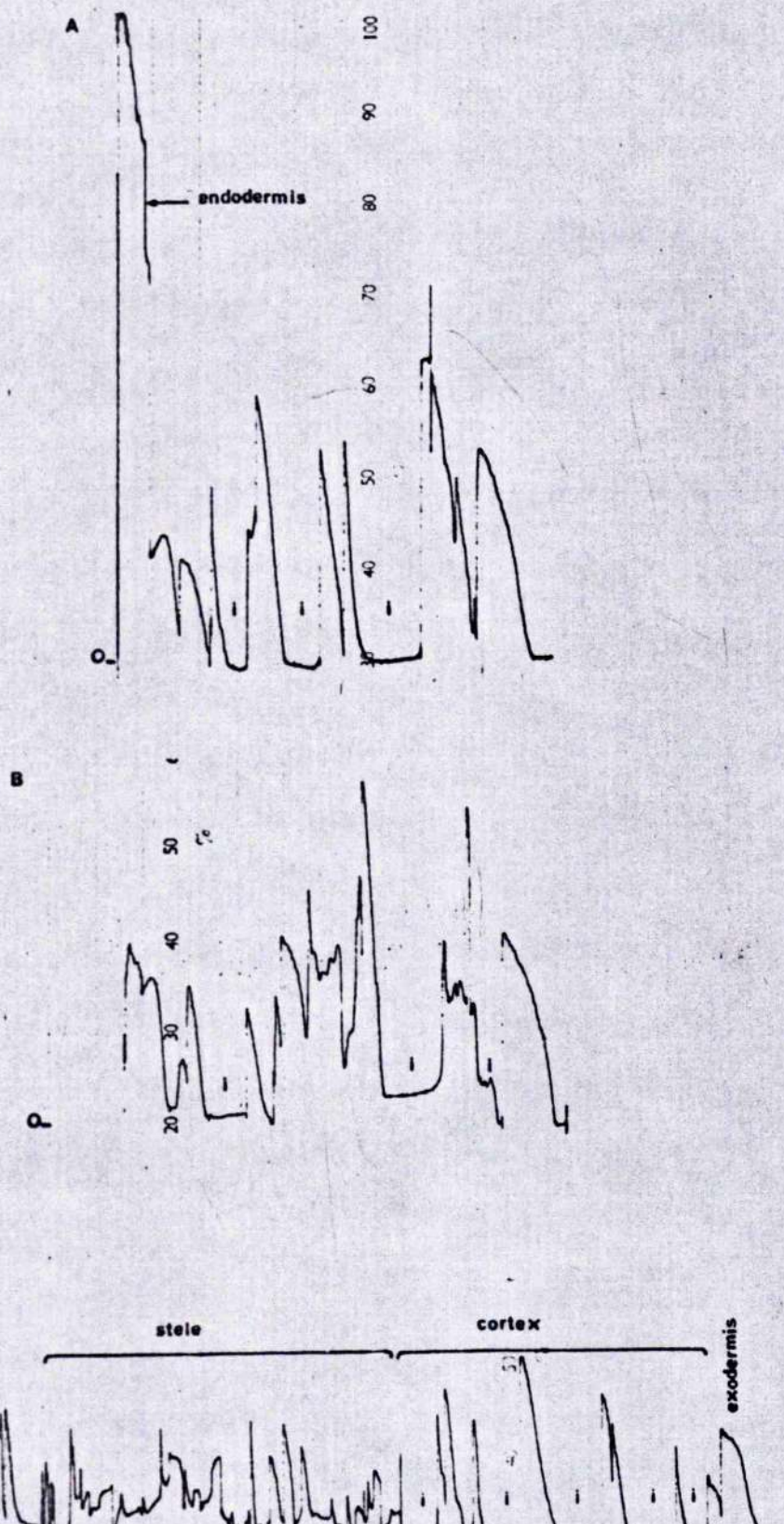
It was therefore decided that statistically comparable data could be obtained by repeated penetration of the root by an electrode, in the regions 4-6 in Figure 22, and that recordings could be made over a 2 hour period following solution change.

The snag with this approach was that a complete recording potentials across the stele could not be made, as the electrode would traverse the root above the stelar tissues. It was anticipated, therefore, that only occasional records of endodermal potentials would be obtained.

The saline used as a basis of these and future experiments was made up according to Pringsheim (1951) from

Figure 24

Effect of increase in sea water concn. on cell potentials



Smith (1951), with a composition of 520 mEq/ Na/litre, 130 mEq.K/litre, and 650 mEq.Cl/litre. Unfortunately, it was not realised until much later, when routine analysis of sea water from the Eden estuary were made, that the value quoted for K in the reference was 10 times in excess of the correct value. At this point the experiments were continued as the results were not invalid inasmuch as they referred to a standard solution, but with a higher concentration both with respect to K, and overall, than sea water. The term 'artificial sea water' will be retained in description.

c) The effect of increase in concentration of artificial sea water on exodermal and cortical potentials

It was decided to perform an experiment whereby the sea water concentration was increased from 0 - 1/10 - 1/5 - ½ - 1, by volume. A series of penetrations across the root to the stele were made at 10 minute intervals for 60-90 minutes after each change. The mean exodermal and cortical potentials were plotted against concentration by volume, and independently against the log. concentration of each ion present.

Figure 24, traces A ad B show examples of the stability of the potentials with time after increase in concentration from 1/5 - ½ sea water concentration. Trace A was made 15 minutes after solution change, and trace B 132 minutes after the change. The values of these exodermal and cortical potentials are markedly higher than those of trace C, which is a record taken 33 minutes

after change from ½ to full artificial sea water concentration.

It is interesting to note that there was no potential in traces A and C between the intercellular spaces (i) and the bathing solution, but that slight potentials were observed in trace B, 2 hours after solution change.

The endodermal potential of -70 mV is recorded on trace A and can be seen to be much greater than the values for cortex and exodermis. It would appear that at this concentration the endodermis is an effective barrier against free movement of ions. Unfortunately trace B shows no record of penetration of the endodermis. However, trace C, does traverse most of the stele and it is very obvious that the permeability barrier at the endodermis has ceased to be operative. The electrical potentials which were recorded from the stelar tissues are even lower than those of the cortex.

The mean potentials together with the Standard Deviation from the mean for the electrical potential recorded in this experiment are shown in table 4.

Figure 25 A

Mean potentials with increasing sea water concentration

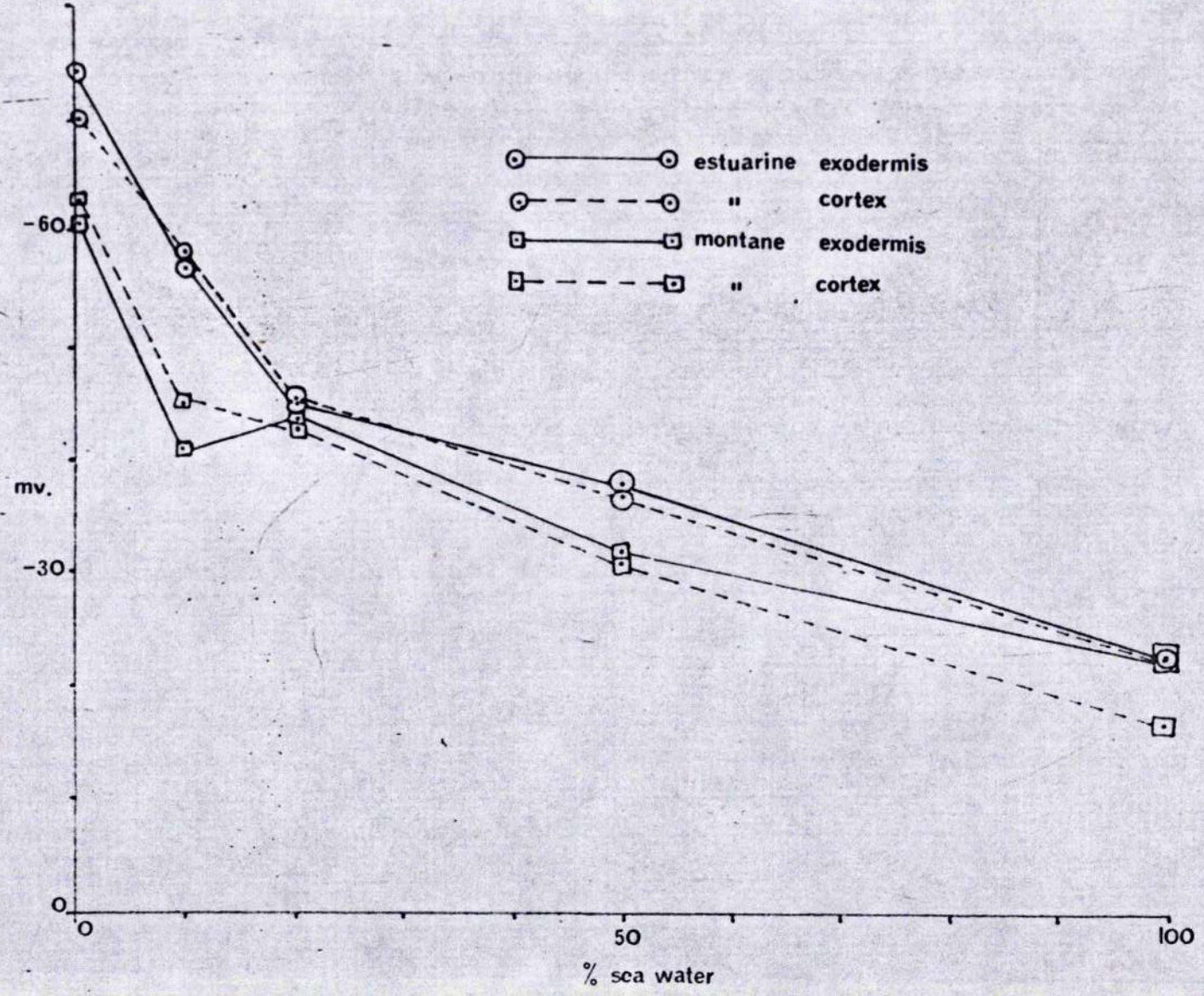


Figure 25 B

Mean estuarine exodermal potential vs. log. ion concentration

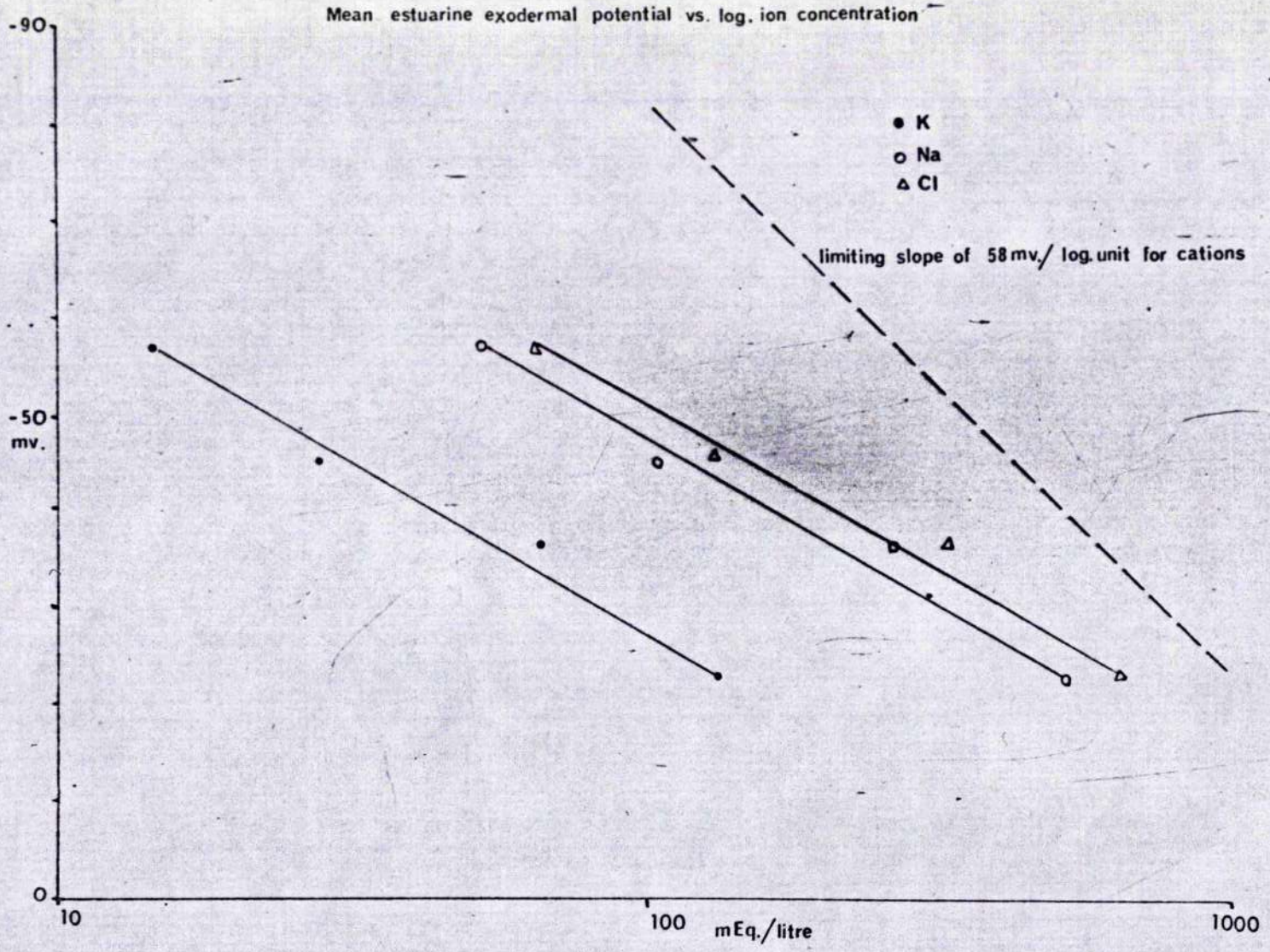


Figure 25 C

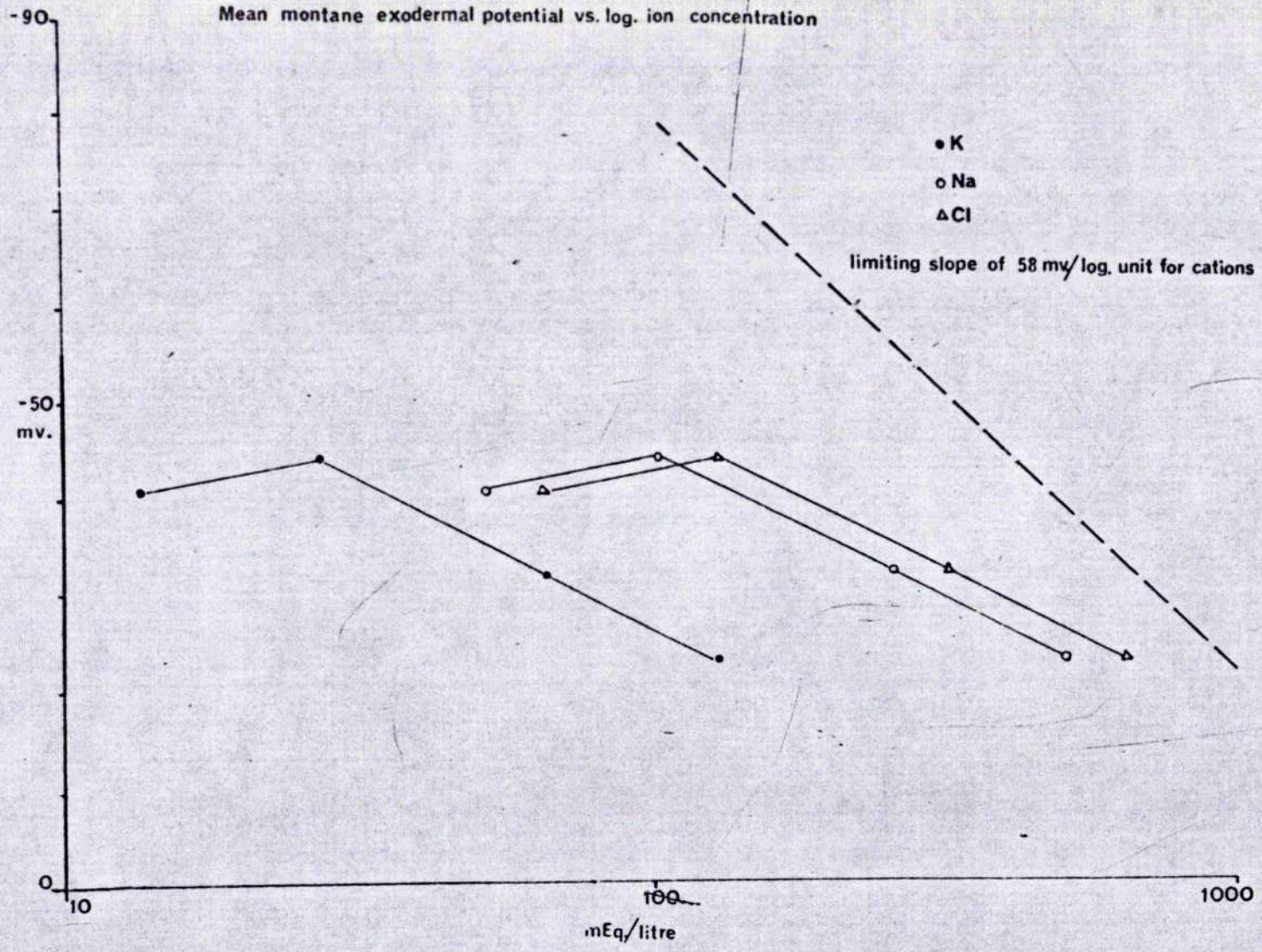

Mean montane exodermal potential vs. log. ion concentration
-90
-50
mv.
0
10
100
1000
mEq/litre
• K
o Na
Δ Cl
limiting slope of 58 mv/log. unit for cations

Table 4.

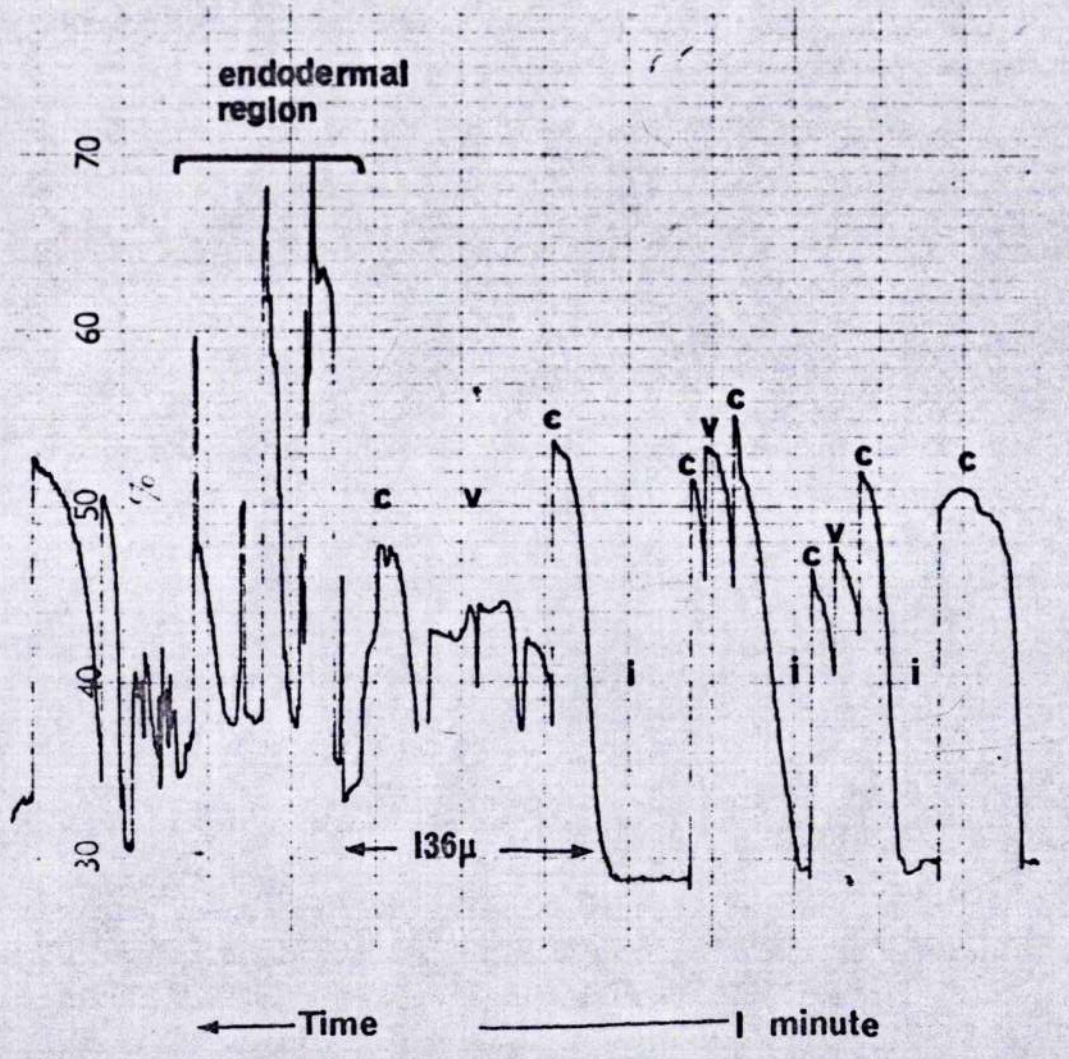
Concentration	Exodermis		Cortex	
	Estuarine	Montane	Estuarine	Montane
normal culture	-74^{+} 3mV	-61^{+} 4.8mV	-70^{+} 6.5mV	-63^{+} 5.8mV
1/10	-57^{+} 10.5	-41^{+} 8.8mV	-57^{+} 2.8mV	-45^{+} 7.7mV
1/5	-47^{+} 8.3	-44^{+} 5.4mV	-47^{+} 6.2mV	-33^{+} 5.7mV
1/2	-38^{+} 12.4	-32^{+} 4.3mV	-37^{+} 9.5mV	-31^{+} 3.1mV
1	-23^{+} 3.2	-23^{+} 5.7mV	-23^{+} 3.5mV	-17^{+} 3.8mV

The values for decrease in negative potential with increase in concentration of Cl, recorded in 25B, and C, are more meaningful when interpreted as being decrease in potential with increase in total cation concentration.

The results are plotted in Figures 25A, B and C.

Application of the t-test showed no statistical difference at the 95% limit, between the means of the exodermal and cortical populations, except between the montane values at the highest concentration. In general, however, the means of the exodermal potentials appeared to be higher than those of the cortex as the concentration was increased. The means of the estuarine and montane exodermal potentials lie within the same population, with the

Figure 26



exception of the values following the introduction of 10% sea water. The cortical means show different populations after 10% and complete sea water, but are similar over the 20 - 30% range.

d) <u>The effect of variation of the Na : K ratio on exodermal and cortical potentials at the concentration of artificial sea water</u>

The experiment was performed in the same manner as that described above (c) except that the overall concentration was that of artificial sea water, but the ratio of Na:K was changed from 1:4 through 2:3 and 3:2 to 4:1.

A typical trace through the root obtained in artificial sea water with a 4 Na : K ratio is shown in figure 26. The electrode appears to have passed through the cytoplasm only, of the exodermis, and through the cytoplasm, the peripheral region of the vacuole and the cytoplasm of the first and second cortical cells, before passing along the complete diameter of 136µ of the innermost cortical cell. The last interpretation may not be correct but the time scale suggests that it could be so. It is probable that most of the higher values of up to -40 mV, correspond with the endodermis, as it was unlikely that plane of penetration as explained in Section 3, b, would allow the full diameter of the stele to be traversed.

The results are shown in table 5, and are plotted in Figure 27.

Figure 27

Mean potentials with change in K:Na ratio at sea water concentration

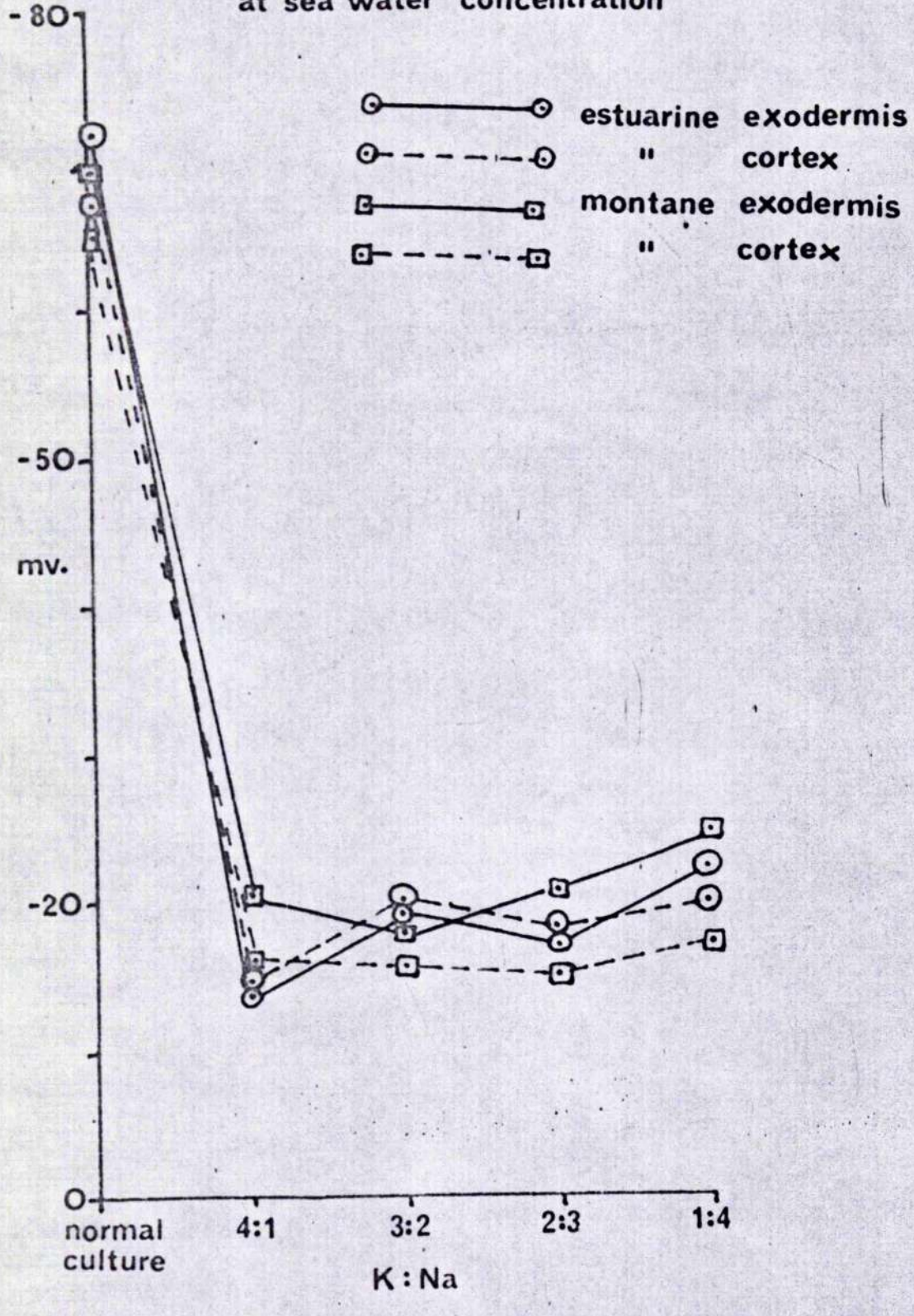


Table 5.

	Exodermis		Cortex	
	Estuarine	Montane	Estuarine	Montane
Culture	-72^{+}_{-} 6.8mV	-69^{+}_{-} 7.1mV	-66^{+}_{-} 5.7mV	-67^{+}_{-} 6mV
Ratio				
1 Na:4K	-14^{+}_{-} 4mV	-21^{+}_{-} 4.6mV	-15^{+}_{-} 4.4mV	-16^{+}_{-} 1.7mV
2 Na:3K	-19^{+}_{-} 4.8mV	-18^{+}_{-} 6.2mV	-20^{+}_{-} 1.8mV	-16^{+}_{-} 3.9mV
3 Na:2K	-17^{+}_{-} 3.7mV	-21^{+}_{-} 1.8mV	-18^{+}_{-} 3.1mV	-15^{+}_{-} 1.5mV
4 Na:1K	-22^{+}_{-} 5.6mV	-24^{+}_{-} 4.8mV	-20^{+}_{-} 4.3mV	-17^{+}_{-} 2.8mV

Application of the t-test to the results, showed a significant rise in the negative potential of the exodermal and cortical cells, from the extreme of high K to the extreme of high Na. There was no significant variation between adjacent results. No statistical difference occurred between any pair of montane values, although the tendency was towards a rise in potential, similar to the estuarine root behaviour. Comparison of the mean exodermal and cortical potentials at each ratio value yielded no significant difference in estuarine plants, and at the 2 high K values for montane. At the high Na values the montane exodermis recorded significantly more negative values than the cortex. Comparison of the exodermal potentials revealed a significant difference of the means with

1 Na:4K and 3 Na:2K, similar treatment of the results for cortical potentials showed difference of the means only at 2Na:K ratio in the artificial sea water.

e) Discussion

(i) Permeability barriers

As a result of the experiments described in this section, the nature of the permeability barriers can be commented upon. Increase in the concentrations of the bathing medium resulted in loss of the intercellular space potentials. A corresponding fall of -20 mV in the electrical potential, recorded between the endodermis and the bathing solution also occurred. This fall in potential is of the same magnitude as the total intercellular space potential, before the concentration of the ambient medium was increased. (Figures 15 and 24). The negative values of the exodermal and cortical potentials were themselves reduced.

Further contrast between the exodermis and endodermis was provided by their behaviour on repeated penetration by an electrode. Withdrawal of an electrode results in the perforation of the tissues through which it had been driven. It might be expected that successive penetration of a barrier to diffusion, would result in the potential previously recorded inside the tissue, becoming short circuited through the adjacent perforation. Repeated perforation of the exodermis, when the root was bathed in normal culture, did not reduce the value of the intercellular

potential. However, a second puncture of the endodermis resulted in a fall of -60 mV compared with the potential recorded from this structure, and some cells to its interior, in the first recording. This fall was consistently achieved whether the external concentration had increased, (Figure 24C), or not. Comparison with Figure 26 shows that the effect is one of perforation and not of concentration.

It must be concluded from this evidence that at least a part of the stelar volume within the endodermis behaved as if it were a single cell, the endodermis being equivalent to a bounding membrane. Upon repeated puncture, the potential across this barrier was short-circuited. The endodermis, therefore, acts as a permeability barrier through a function of the protoplasts of this tissue, to regulate ion movement to the stele.

In contrast, the potential between the intercellular spaces and the ambient medium, or between the cortical cells and the medium was not reduced on repeated puncture, but did fall on increase in concentration of the bathing medium. This evidence fits a model of passive entry into the root, controlled by different ion mobilities in the tissue; that is, the intercellular potential was Donnan in origin. Further evidence of this can be seen by comparing the apparent width of the intercellular spaces, recorded on the traces shown in Figure 15 and 24A. The intercellular spaces recorded on the latter trace

appear to be wider than those of Figure 15. This effect was constant in other recordings: and can be explained on the basis of a decrease in the negative value of the electrical potential, with corresponding increase in distance from the site of the fixed anions causing the Donnan potential. (Dainty and Hope 1961). Thus a gradual, rather than a sudden, fall in potential is recorded on emergence of the microelectrode tip into the intercellular space. Similarly, the increase in negative potential, begins gradually prior to cell penetration, rather than as a sudden effect. Increase in the exodermal concentration causes loss of the Donnan potential in the intercellular spaces. Similarly, penetration of, and emergence from a cell by an electrode, is recorded as a sharp change in potential, and the apparent width of the intercellular spaces is increased.

However, continued exposure of the root to "½ sea water", led to the potential in the intercellular spaces re-appearing. This tendency was recorded on several occasions, at both this, and lower external concentrations. The recovery generally began sooner with more dilute bathing media (1/10 and 1/5). The recovery was not observed with artificial sea water at full concentration, but in this case sufficient time might not have elapsed before the recordings were stopped.

This recovery cannot be explained on the Donnan hypothesis. Hence, it might be necessary to invoke a mechanism of combined control of the potential, by the exodermis and

cortical cells. This would operate once initial rapid ion movement into the cortex, from a bathing solution whose concentration had suddenly increased, has ceased. Export of Na from the root, together with higher permeability of cell membranes to K than Cl, may result in a negative intercellular potential becoming re-established.

(ii) Effects on membrane potential of changing cation ratios at constant external concentration

Figure 25A shows 2 linear phases for change in potential with increase in external concentration, the first of which corresponded with the range of concentration values, over which relative permeability values were calculated, (Section 2). It would seem that at higher concentrations the relative values changed. Semi-logarithmic graphs for individual ion concentrations (Figures 25B and C) were constructed.

It would appear from these graphs, that at high saline concentrations the negative electrical potential recorded at the exodermis, reduced exponentially with increase in saline concentration. This can be explained on the basis of a change in the permeability of the exodermis to the major ions, such that the anion, Cl, became relatively more permeable. The steady value recorded in 25C, at intermediate (200 to 300 mEq/l. total cation) concentrations, may also be

explained on the basis of an increase in Cl permeability. However, a sudden decrease in the cation permeability may also have occurred. This would have the effect of radically increasing the relative Cl permeability, so maintaining the steady potential over this intermediate range of external concentrations. It was considered possible at this stage that active accumulation of a cation by montane roots, may cease over the concentration range of 200-300 mEq/l. of cation. Further decrease in electrical potential along an exponential gradient, which had the same slope on a semi-logoritimic graph, as for an estuarine root, may be attributable to passive inward movement of salt. The relative rates of this movement would be comparable in roots from estuarine and montane plants.

It was hoped that data obtained from the effect of changes in concentrations and cation ratios, on the electrochemical gradient for each ion, between leaf and solution, would help to elucidate these points.

If it is assumed that immediately following increase in external concentration, the concentration of each ion in the exodermal cells was negligible, then values for the electrical potential between cell and bathing medium can be calculated from the Goldman equation, if the relative

permeabilities are known.

On this basis, and using the values for relative permeabilities obtained from Section 2, the values predicted for the electrical potentials between endodermis and concentrated bathing solutions, having cation ratios of:- Na:4K and 4Na:1K are:-

	Estuarine	Montane
1Na:4K	+48mV	+21mV
4Na:1K	+35mV	+12mV
E	+13mV	+ 9mV

The values actually recorded were:-

	Estuarine	Montane
1Na:4K	-14 $\pm$ 4mV	-21 $\pm$ 4.6mV
4Na:1K	-22 $\pm$ 5.6mV	-24 $\pm$ 4.8mV
E	-8mV	-3mV

Obviously, the results are not quantitatively comparable as some increase in internal ionic concentration will occur during the 10 minutes before recordings were taken. Also the relative permeabilities have been shown to change, although no quantitative values could be obtained from the data.

However, the results obtained from the experiment do show variation between the potentials at high and low Na:K ratios. These variations are of the order of those expected, and are statistically significant for estuarine roots.

The proposed apparent increase in relative permeability of the membranes to Cl would, if real, reduce the difference between the recorded potentials at high and low Na:K, as was found.

It was suggested on this basis, that use of values for relative permeabilities found in Section 2, would not, when combined with measurements of ion concentrations, yield accurate predictions for electrical potential measured between leaf and bathing medium. However, the predicted potentials would be expected to be of the correct order of magnitude, especially at low external concentrations, if the exodermis, or a membrane showing similar permeability propoerties, controlled movement of ions into the xylem sap.

Salt balance in the whole plant

Section 4.

a) Determinations of the electrochemical potentials of Na:K and Cl in the leaf W.F.S., with varied ion concentrations in the ambient medium

As has been stated in Chapter 1., the basic aim of this research was to provide data with an electrochemical basis, to explain salt regulation in Armeria maritima. Experiments which have been described previously have shown the existence of permeability barriers to free ion entry in the root. The membranes of the root exodermal cells appear to be relatively more permeable to Cl than to the cations, at higher concentrations of the bathing medium.

It was hoped that change in the electrochemical potentials of the ions in the W.F.S.(xylem sap) with increase in external ion concentrations, would produce data which could be interpreted in terms of results already described.

Salt glands situated in the leaves of Armeria maritima have been described as de-salinators (Montfort, 1922), but their efficiency in maintaining a tolerable level of salt in the plant has not been assessed.

It was proposed to relate data obtained from this experiment, to the relative efficiency of the root and glands,

in maintaining a steady internal ionic environment.

Method

The concentrations of ions in the leaf W.P.S. were determined using the techniques described in Chapter 2; Sections 3, 4 and 5. The electrical potential was measured as described in Chapter 2, Section 6.

Each pair of plants, one estuarine and one montane in origin, was subjected to increase in concentration of the bathing medium from normal culture to 1/10, then 1/5, ½, and finally to that of artificial sea water. Four basic solutions were used, having cation ratios of 1Na:4K, 2Na:3K, 3Na:2K and 4Na:1K. This enabled the effect of a wide range of external cation concentrations to be studied, and afforded the opportunity of detecting any effect caused by high Na as distinct from high K.

The conditions of ions obtained from both the free space and medium, were fitted to the Nernst equation and the potential calculated for each ion compared with the observed electric potential. To fulfill the criteria on which the Nernst equation is based, the ions in the compartments studied must be in flux equilibrium. The likelihood of this situation occurring between the widely separated phases of the leaf free space and the bathing medium is small, as to attain this state, all the tissues along, and affecting the transport pathway,

must themselves be in equilibrium. Growth or senescence of any tissue will cause the system to leave equilibrium. In an attempt to reduce these effects, the experiments were carried out in late September, and on 'heads' deemed to be "mature" on visual examination. It was hoped that at this time of year growth would be minimal and the ageing of leaves which became apparent visually in late December, would not have begun.

When normal culture was replaced by artificial sea water, the time taken for the electrical potential across the plant to drop from -75 mV to a steady value of -40mV was 192 minutes; 60 minutes elapsing before the effect of change of solution became detectable. The reverse process took 204 minutes; 120 minutes being the time lapse before a detectable effect was noticed. The potential recovered its original value. It was adjudged that electrical equilibrium had been reached during this time, due to initial fluxes of one ion, or ions of one sign, being much greater than those of the other sign; a process which is followed by adjustment of the ion pairs slowly with time.

It was decided on the basis of the relatively rapid electrical adjustment, the suspected slight difference in relative permeabilities, and the shortage of time left for experimentation, to allow 3 days to elapse for the adjustment of partial ion fluxes towards flux equilibrium.

TABLE 6

SOLUTION	CONCN.	ESTUARINE							MONTANE						
		E obs.	E calcd.			E obs. − E calcd.			E obs.	E calcd.			E obs. − E calcd.		
			Na	K	Cl	Na	K	Cl		Na	K	Cl	Na	K	Cl
CULTURE	NORMAL	−110 mv.	—	—	+17 mv.	—	—	−127 mv.	−77 mv.	—	−165 mv	—	—	+88 mv	—
1Na : 4K	$\frac{1}{10}$	−66 "	−55 mv.	−73 mv.	+38 "	−11 mv.	+7 mv.	−104 "	−70 "	−82 mv.	−88 "	—	+12 mv.	+18 "	—
"	$\frac{1}{5}$	−58 "	−27 "	−44 "	—	−31 "	−14 "	—	−60 "	−25 "	−61 "	+75 mv.	−35 "	+1 "	−135 mv.
"	$\frac{1}{2}$	−38 "	+13 "	−20 "	+21 "	−51 "	−18 "	−59 "	−62 "	−14 "	−41 "	+41 "	−48 "	−21 "	−103 "
"	1	−56 "	−40 "	−39 "	+36 "	−16 "	−17 "	−92 "	−32 "	+2 "	−32 "	+20 "	−34 "	0 "	−52 "
CULTURE	NORMAL	−91 "	—	−153 "	+226 "	—	+62 "	−317 "	−80 "	—	−184 "	+198 "	—	+104 "	−278 "
2Na : 3K	$\frac{1}{10}$	−68 "	−58 "	−99 "	—	−10 "	+31 "	—	−66 "	−25 "	−79 "	+104 "	−41 "	+13 "	−170 "
"	$\frac{1}{5}$	−68 "	−45 "	−101 "	+94 "	−23 "	+33 "	−164 "	−60 "	−5 "	−68 "	+57 "	−55 "	+8 "	−117 "
"	$\frac{1}{2}$	−40 "	−46 "	−49 "	+48 "	+6 "	+9 "	−88 "	−44 "	−15 "	−57 "	+31 "	−29 "	+13 "	−75 "
"	1	−44 "	−32 "	−51 "	+44 "	−12 "	+7 "	−88 "	−52 "	+15 "	−35 "	+12 "	−67 "	−17 "	−64 "
CULTURE	NORMAL	−80 "	—	−159 "	+197 "	—	+79 "	−277 "	−78 "	—	−175 "	—	—	+97 "	—
3Na : 2K	$\frac{1}{10}$	−65 "	−42 "	−100 "	+105 "	−23 "	+35 "	−170 "	−68 "	−44 "	−124 "	+104 "	−24 "	+56 "	−172 "
"	$\frac{1}{5}$	−68 "	−42 "	−69 "	+54 "	−26 "	+1 "	−122 "	−64 "	−23 "	−83 "	+82 "	−41 "	+19 "	−146 "
"	$\frac{1}{2}$	−42 "	−22 "	−68 "	+51 "	−20 "	+26 "	−93 "	−40 "	−12 "	−56 "	+39 "	−28 "	+16 "	−79 "
"	1	−62 "	−14 "	−42 "	+36 "	−4 "	+19 "	−111 "	−78 "	+6 "	−56 "	+36 "	−84 "	−22 "	−114 "
CULTURE	NORMAL	−100 "	—	−160 "	—	—	+60 "	—	−70 "	—	−180 "	—	—	+110 "	—
4Na : 1K	$\frac{1}{10}$	−54 "	−40 "	−128 "	+79 "	−14 "	+74 "	−133 "	−63 "	−27 "	−123 "	+94 "	−36 "	+60 "	−157 "
"	$\frac{1}{5}$	−45 "	−7 "	−90 "	+54 "	−38 "	+45 "	−99 "	−70 "	−27 "	−98 "	+61 "	−43 "	+28 "	−131 "
"	$\frac{1}{2}$	−40 "	−13 "	−64 "	+52 "	−27 "	+24 "	−92 "	−66 "	+6 "	−83 "	+38 "	−72 "	+17 "	−104 "
"	1	−54	−50 "	−73 "	+57 "	−4 "	+19 "	−111 "	−57 "	+11 "	−66 "	+22 "	−68 "	+9 "	−79 "

Figure 28 A

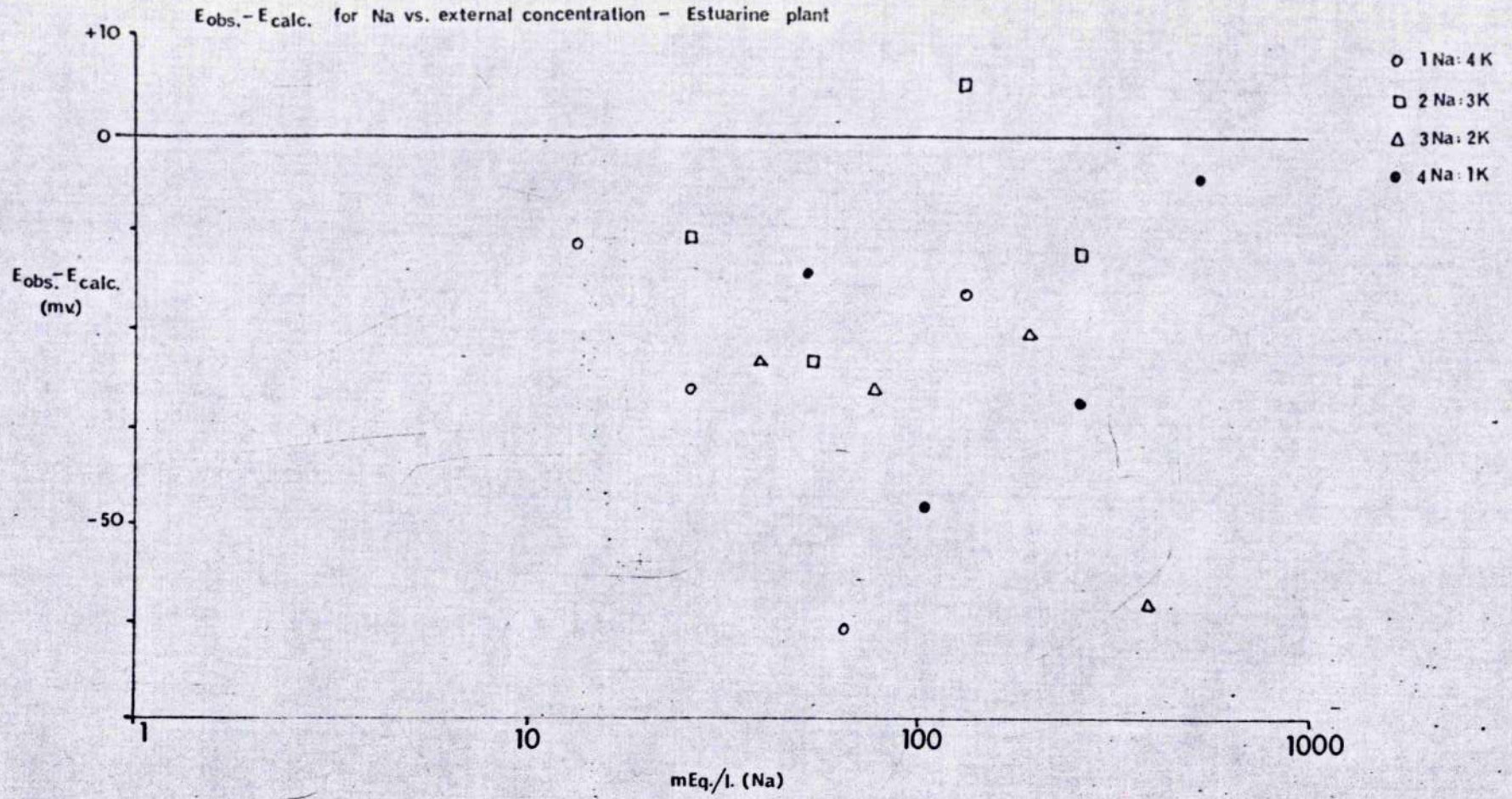

$E_{obs.} - E_{calc.}$ for Na vs. external concentration – Estuarine plant
+10
0
-50
$E_{obs.} - E_{calc.}$ (mv)
1
10
100
1000
mEq./l. (Na)
1 Na : 4 K
2 Na : 3K
3 Na : 2K
4 Na : 1K

Figure 28 B

$E_{obs.} - E_{calc.}$ for Na vs. external concentration – Montane plant

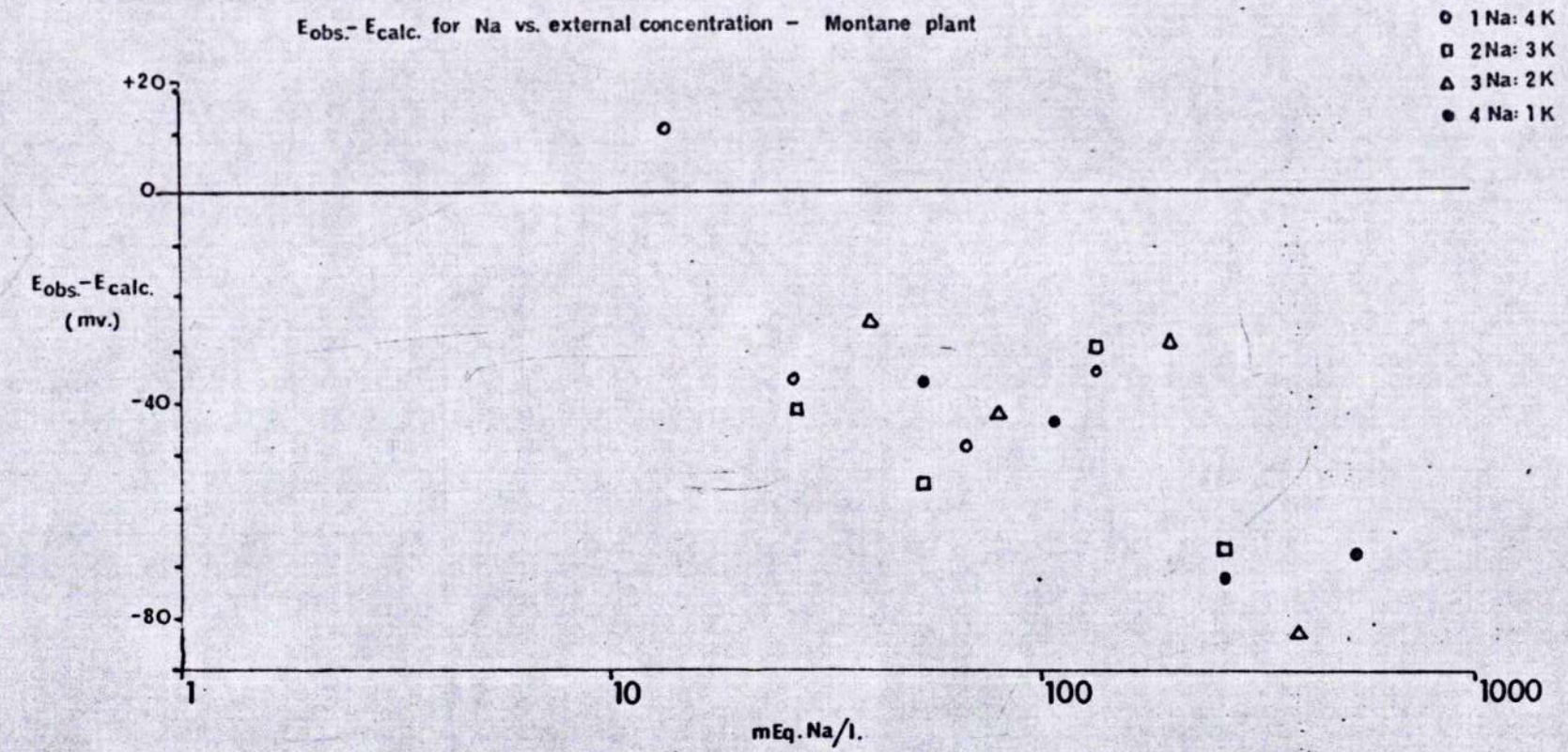


Figure 28C

$E_{obs.} - E_{calc.}$ for K vs external concentration – Estuarine plant –

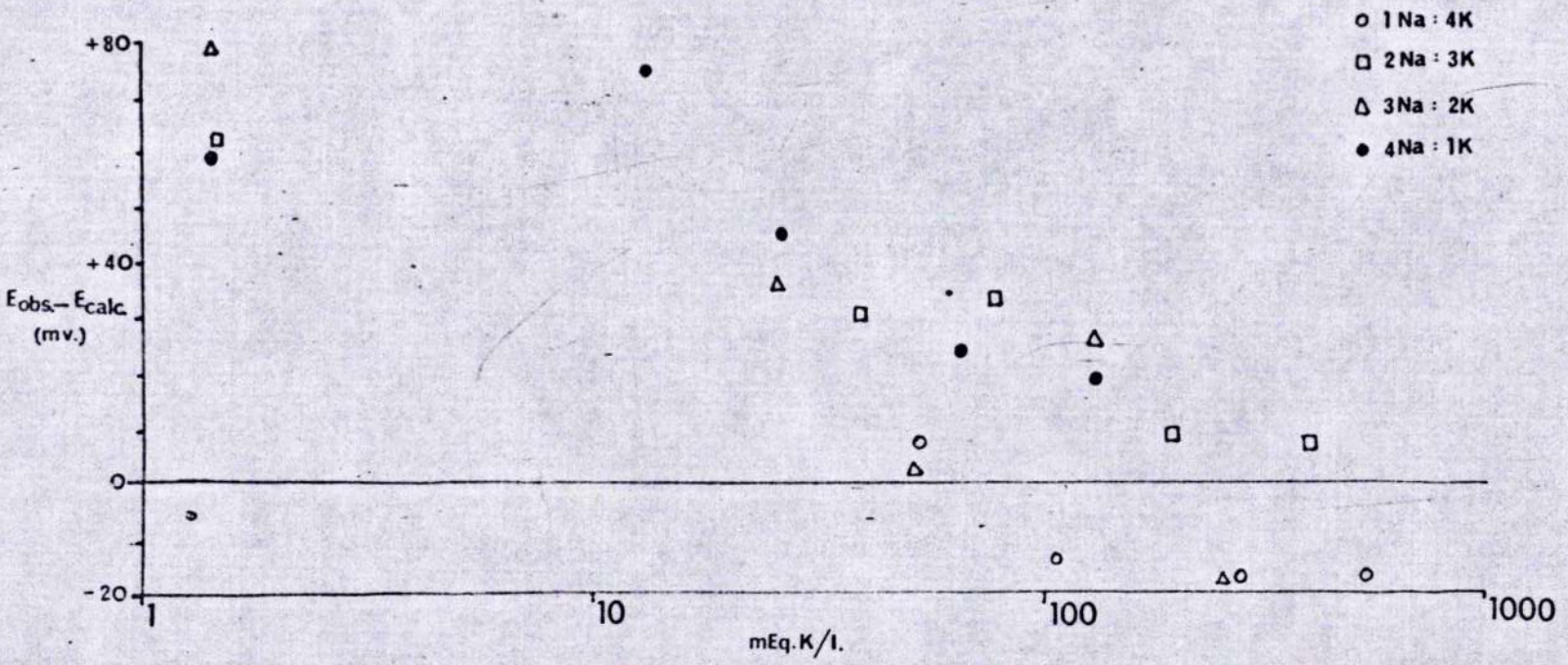


Figure 28 D

$E_{obs.} - E_{calc.}$ for K vs. external concentration – Montane plant

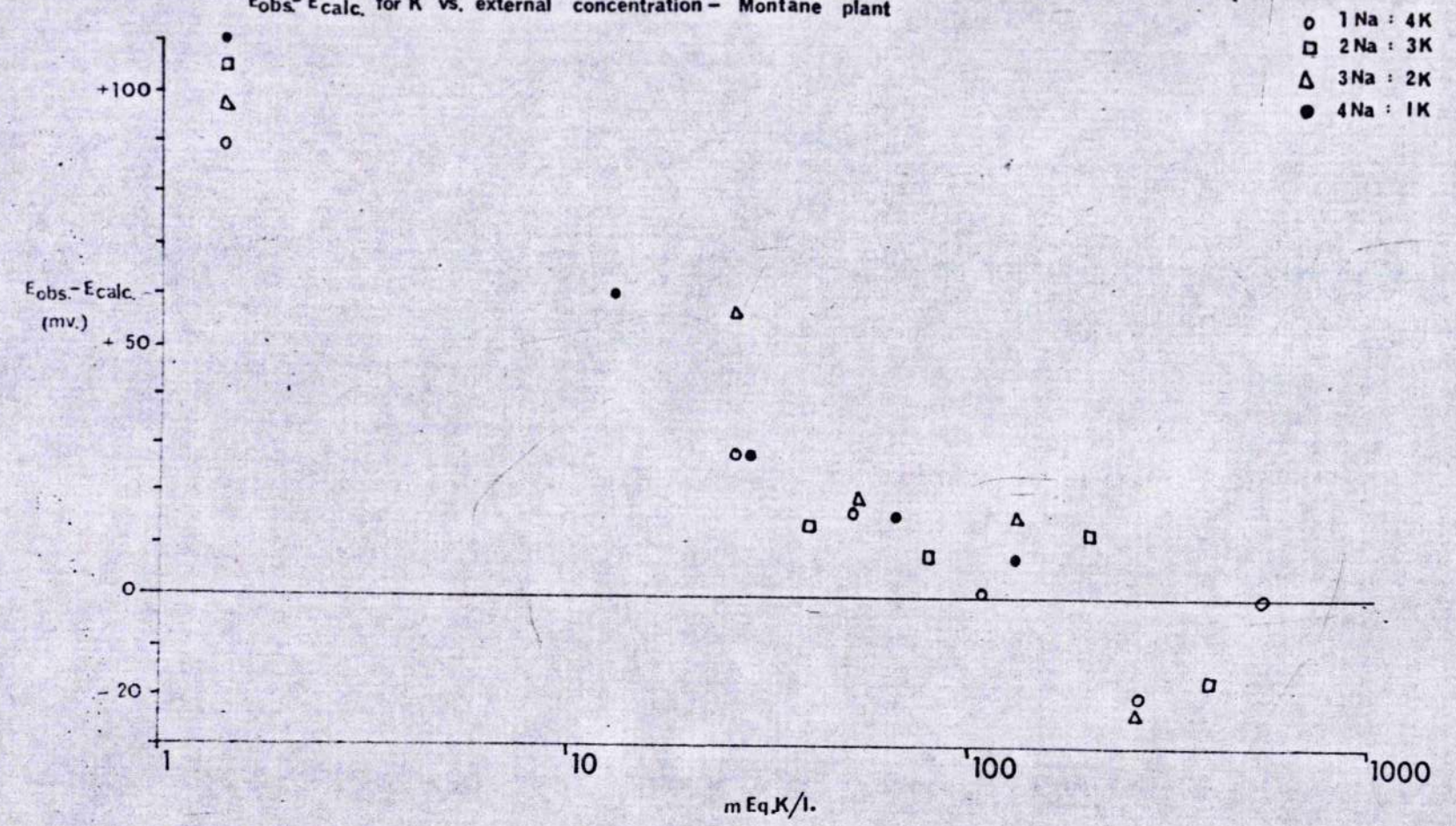
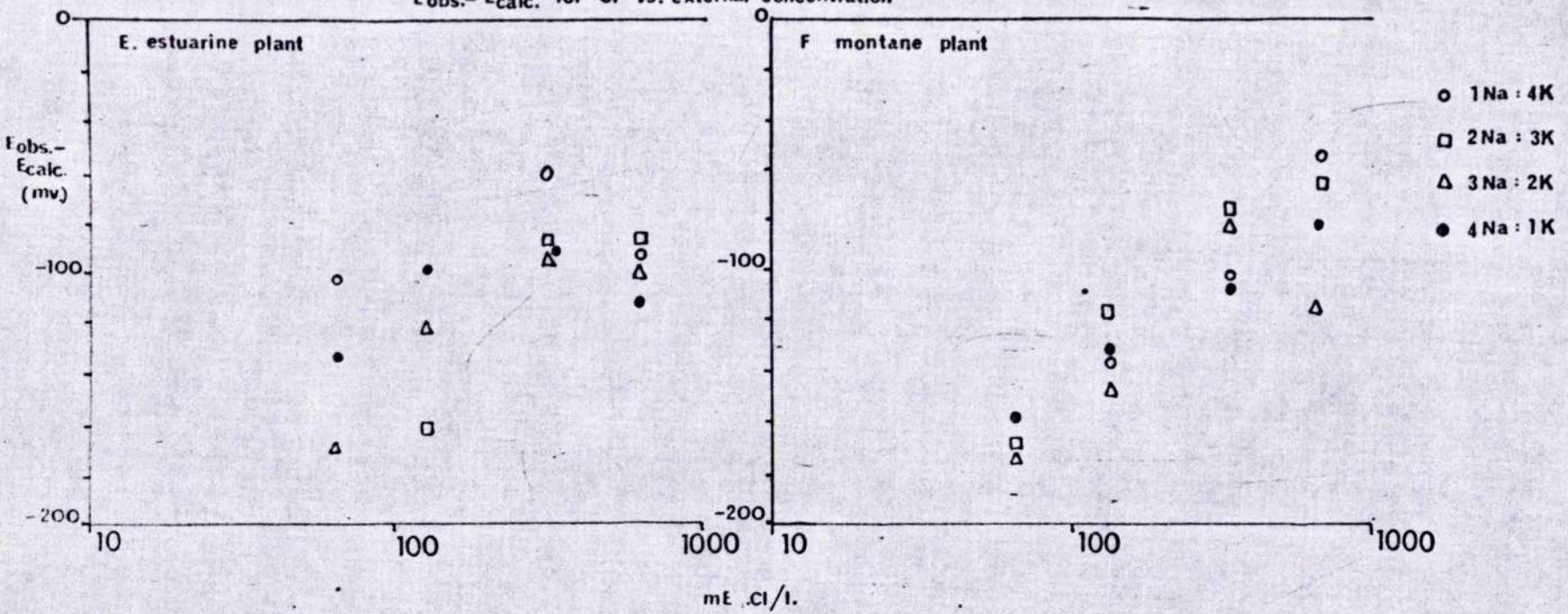


Figure 28

$E_{obs.} - E_{calc.}$ for Cl vs. external concentration



The leaves sampled in each experiment were the leaves from which the potential had been measured, so the potential calculated for each ion, (E calcd.) could be validly compared with results that measured (E obs.). The air temperature throughout the course of the experiment varied from 16-19°C.

Results The results obtained are tabulated in Table 6, and the values for E obs - E calcd. with external concentration are expressed graphically in Figures 28A - F. For cations, a positive value of E obs - E calcd. is indicative of active accumulation, and a negative value of active excretion. Similarly for Cl, a negative value is indicative of accumulation against an electrochemical gradient. The numerical value of each point corresponds to a quantitative value for the forces acting on the individual ions, providing flux equilibrium has been attained.

Point values only are recorded on the graphs, the different symbols representing the various cationic compositions of the bathing media.

The data presented in Figures 28 A-F shows: excretion of Na; accumulation of K at low concentrations, with a tendency towards passive movement, and possibly active excretion, at high and very high concentrations respectively; and active accumulation of Cl. The values for E obs - E calc. for Cl

at low external concentrations, are very negative (table 6). These values were not included on the graphs, as these results were felt to be the least accurate of those obtained. This suspected lack of accuracy was caused by the inaccuracy of the chloride meter at low concentrations. A single unit recorded by the meter was equivalent to a Cl concentration of 0.06 mEq/l in the 3 mls of eluant. The amount of Cl which was eluted at low concentrations of bathing medium was very small, so that an error of 1 unit on the meter was relatively greater than similar errors, produced at higher concentrations.

Therefore, although Cl appeared to be subjected to great passive electrochemical driving force out of the plant at low external concentrations, the values obtained might not have been a true reflection of the magnitude of this potential. Shone (1968), has recorded a similar decrease in electrochemical potential with increase in the concentration of ambient Cl.

Possible effects of non attainment of flux-equilibrium on values for E obs - E calc.

If it is assumed that flux equilibrium has not been established, owing to low permeability of the membranes to the ion in question, for example, (Spanswick and Williams 1965) the value for $\log_{10}$ Co/Ci , which is introduced into the Nernst

equation in its form E = 57.5 $\log_{10}$ Co/Ci at 17°C, will be too high. Reduction of this term as Ci increases at the expense of Co will cause the value of E calculated to become more negative, and E obs - E calc. to become more positive (-E---E). Thus values calculated from the data and found to be in the region of the zero line, should in fact be shifted upwards for a cation, and downwards for an anion ($\frac{Ci}{Co}$). On the other hand, sudden increase in the external concentration of an ion which is normally excreted, may cause an initial influx of that ion along an electrical gradient (for a cation). This ion may not be in flux equilibrium for a reason which is the reverse of that stated above; namely, after initial uptake efflux is greater than influx as the system moves towards equilibrium. $\frac{Co}{Ci}$ is now too small, and at equilibrium the points could be expected to be shifted even more to the negative, with respect to the zero-line.

Possible effects of deviation of the values of activity, from those of concentration of ions in the W.F.S., on the values of E obs. - E calc.

It was thought likely that as ion samples were obtained from the leaf free space by elution, the activity(ai) would be equal to the concentration (Ci) of that ion in the W.F.S. Any deviation from equality would be slight and cause

the true value of $\frac{ao}{ai}$ to be higher than the utilised value of $\frac{co}{ci}$, so causing a shift to the negative for E obs - E calc calculated for cations, using $\frac{co}{ci}$ as the criterion.

Conclusions

From the above predictions, it would seem safe to conclude that at low external concentrations, K was actively transported into the xylem sap in the leaf W.F.S. At higher concentrations the values for E obs - E calc for K, which are in the region of the zero-line, may be shifted towards either active accumulation or active excretion of K, if flux equilibrium has not been attained. The direction and magnitude of this shift would depend on the actual flux ratios.

Active inward transport of Cl from the bathing medium would seem to be a correct interpretation of the data, but the decrease in negative value for E obs - E calc. was possibly due to low membrane permeability to Cl. This would tend to cause an artificially low value for $\frac{Ci}{Co}$, which would in turn yield a less negative value for E obs - E calc. Na appeared to be actively excreted, although some results at high external Na concentrations might indicate swamping of the regulatory mechanism, in estuarine plants. Swamping would occur if the regulatory membranes were sufficiently permeable to Na, to allow passive inward movement of quantities of Na, which were in excess of those which the export mechanisms could remove, whilst pumping at maximum efficiency.

No assessment could be made from the data of the degree of any variance from flux equilibrium. Statistical comparison of point values for E obs - E calc was not valid because of possible effects of the different external cationic ratios, present at each measurement, on the electrochemical potentials. It was possible that there might have been competitive, or inhibitory, interactions between the different ions and the regulatory membranes.

The only valid test for the above conclusions lies in the comparison of predicted values for passive flux ratios for each ion, with the actual flux ratios.

b) Use of ^{22}Na and ^{36}Cl as tracers, in order to determine flux ratios between the xylem and the ambient media.

The experiments were performed as described in Chapter 2, Section 7.

The solutions and plants chosen were selected on the basis of the results shown in Figures 28. It was expected that flux equilibrium had not been attained in this experiment, but that values of E obs - E calc. might be indicative of the ratio of influx : efflux. If this supposition were correct it might be expected that exposure of the root systems of montane plants to sea water at concentrations of 1/10, ½ and 1 by volume, would result in approximately similar flux ratios with the diluted solutions, but that at normal concentrations the flux ratio

Figure 31

Net uptake of ^{36}Cl from $\frac{1}{2}$ by volume artificial sea water

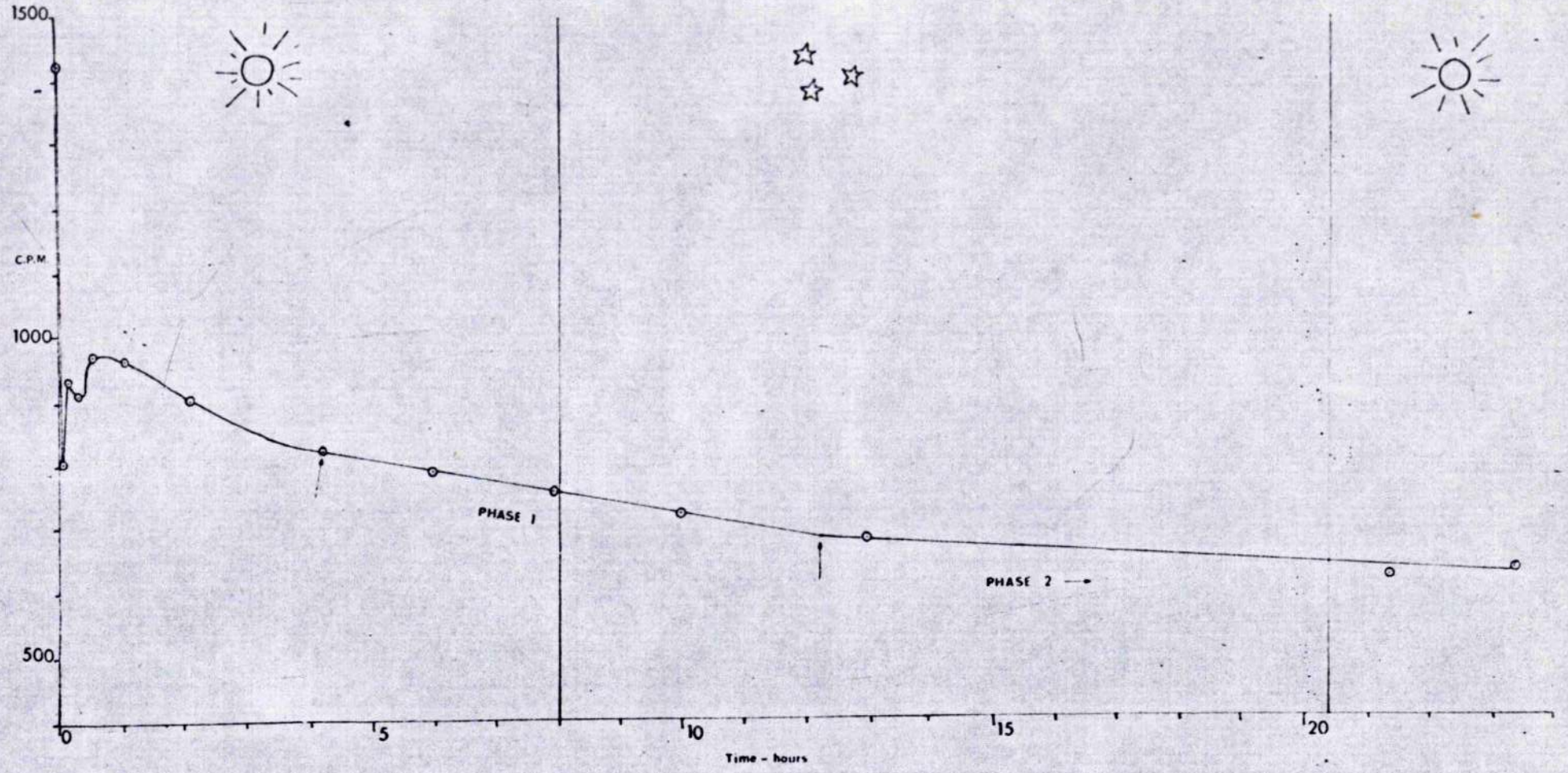


Figure 32

Net uptake of ^{22}Na from artificial sea water

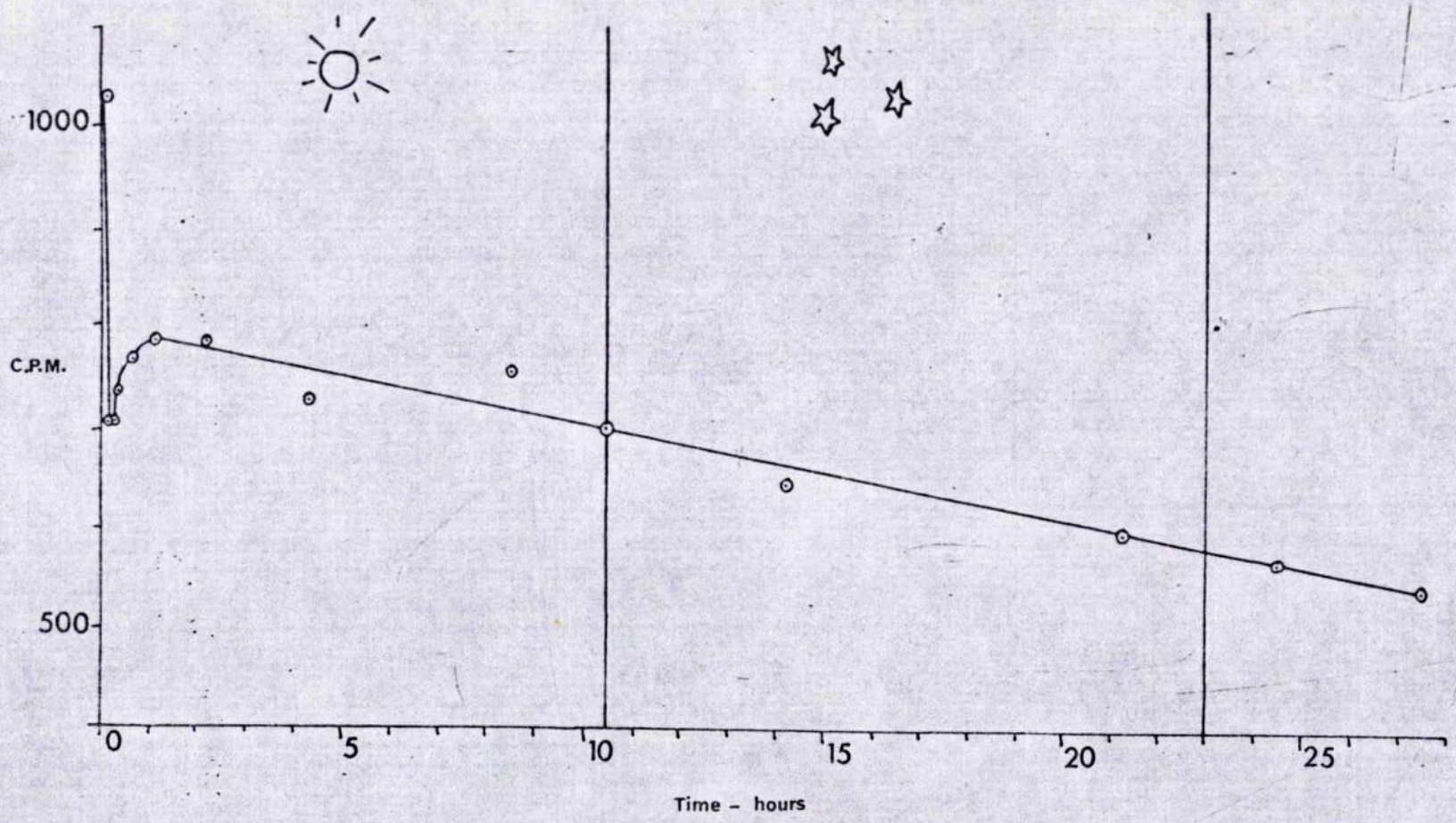
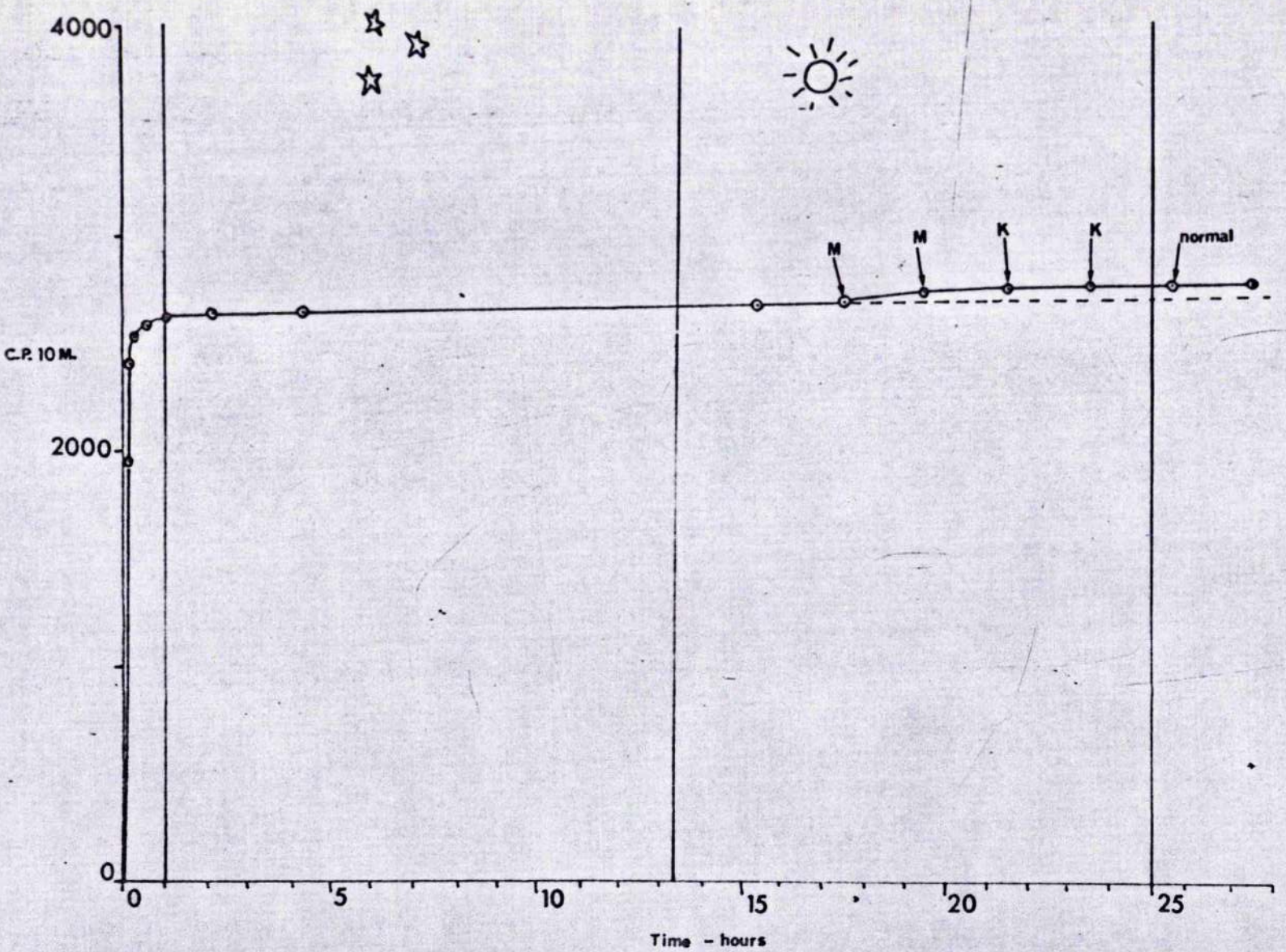


Figure 33

Progress total ^{22}Na lost from roots previously immersed in $^{1}/_{10}$ sea water



calculated using ^{22}Na as a tracer would be lower. This reduced net influx at the higher external concentration would correspond with the more negative values of E obs - E calc., obtained from the previous experiment (Figure 28B).

Similar experiments were planned with estuarine plants using ^{36}Cl to test whether the indication of a greater inwardly directed driving force at sea water concentration compared with ½ sea water concentration provided by Figure 28B would be reflected by the results.

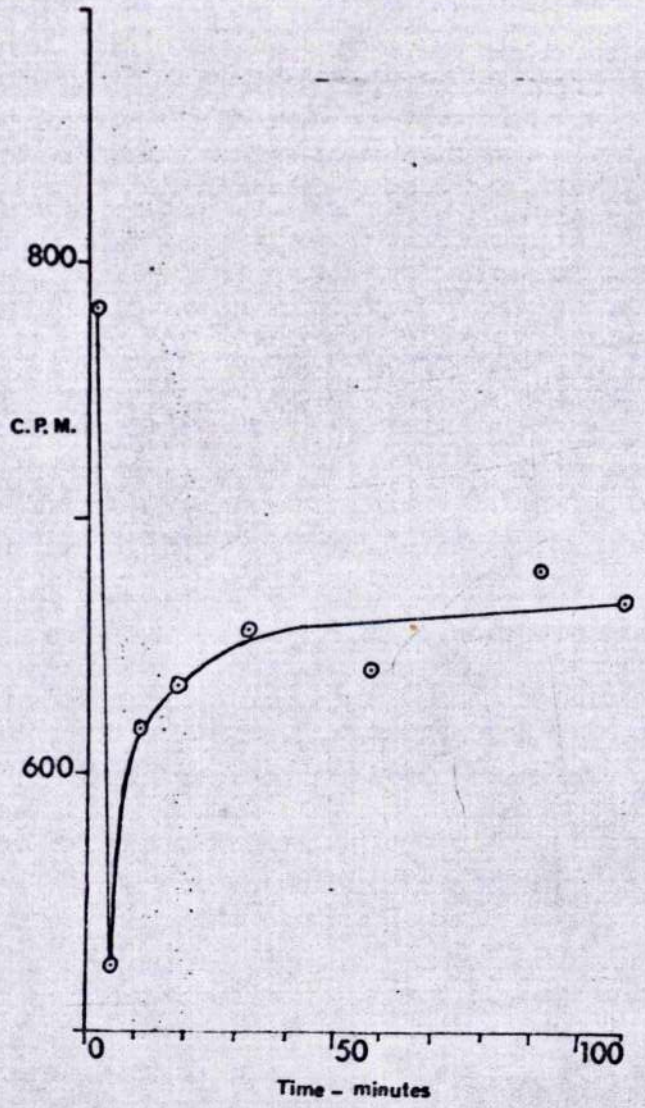
The values obtained for the flux ratios were to be compared with those calculated from the Ussing-Teorell equation using the data previously obtained. Comparison of the results would indicate the existence, and the direction of the driving forces on each ion.

Flux measurement

Net influx was measured as loss of activity from the bathing solution with time. The curves produced with both ^{22}Na and ^{36}Cl were similar and examples are shown in Figure 31 and 32. It will be seen from Figure 31 that uptake of ^{36}Cl appeared to settle into 2 phases, the first steeper phase ending after 12 hours. The time course of uptake of both ^{36}Cl and ^{22}Na showed this bi-phasic system, which was missing from the time courses at high concentration (Figure 32). No sign of a similar bi-phasic system relating to efflux was apparent (Figure 33) at any concentration.

Figure 34
Controls

A. Loss of ^{22}Na from sea water containing dead root tissue



B. Progress total ^{22}Na lost after A

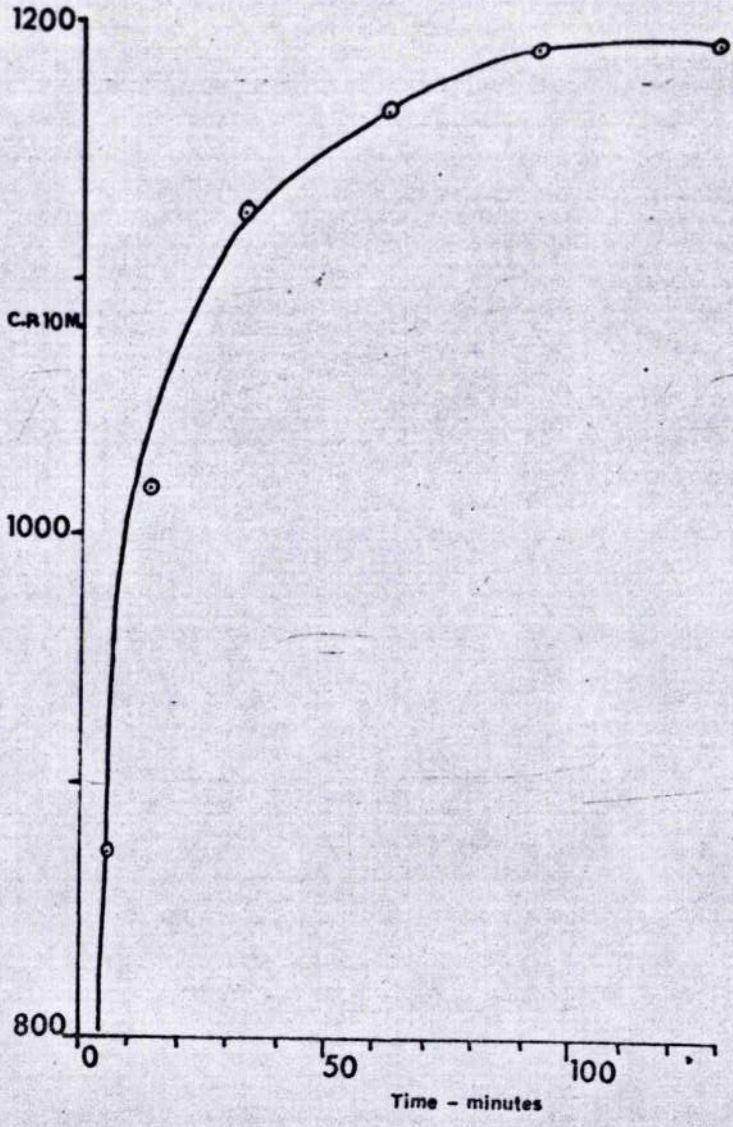


Figure 34 (A & B) represents the uptake and loss of ^{22}Na, using dead root tissue suspended in the apparatus in place of the living plant. On the basis of these curves, it would seem that adsorption and stirring artefacts preclude analysis of influx time courses within the first 2 hours. Similarly, "efflux" measurements over this period would seem likely to include isotope which had been adsorbed on the root surface, and on the glass interior of the converted burette.

It proved impossible to remove and replace the plant, to allow thorough cleaning of the glass vessel between net influx and efflux studies, without damaging the roots and altering the volume, and therefore surface area of the roots exposed to the bathing media. As this was kept constant throughout the experiments, it was possible to compare net influx and efflux directly, without having to correct for changes in the surface area.

Effect of environmental changes.

Light and darkness can be seen to have had no effect on the fluxes, nor did removal of the artificially maintained high humidity. Loss of solution from the apparatus was easily measured using the burette markings, and was of the order of 0.0005 ml./hr./cm^2. rootsurface immersed. The concentration of fluid in the apparatus was maintained by very occasional 'topping up' to the initial level of the solution, using the experimental medium.

Bi-phasic uptake

On the basis of electrical measurements described in Section 5, it is proposed that the bi-phasic uptake observed for ^{22}Na at 1/10, and for ^{36}Cl at 1/10 and 1/5 concentration of volume of artificial sea water, corresponded to uptake by tissues outside the stele. Further uptake was interpreted as being net uptake by the plant tissues.

Possible exchange of Na for K by the root with the bathing medium

In an earlier 'trial-run' performed in normal culture solution, some indication was observed of a possible exchange of K entering the root, and Na leaving it. With this in mind, the efflux experiments were designed so that once a steady rate of ^{22}Na efflux had been obtained, the effect on this rate of introduction of 400 m.eq. K as KNO_3, could be observed. Prior to this addition efflux was carried out in 10 mls. of medium with an added 0.4 mls of 1M. mannitol. This treatment acted as a control to possible effects on efflux rate, caused by increase in volume, and osmotic potential, of the bathing solution.

The arrows in Figure 33 indicate addition of mannitol,(M) and K. It will be noted that increase in efflux occurred, which was associated with mannitol, but not K, addition.

The conclusion from this was that increase in the external K concentration did not affect rate of Na efflux. The increase observed subsequent to mannitol addition was caused purely by increase in the volume of the bathing medium, from

10 to 10.4 mls.

Comparison of actual predicted flux ratios

The fluxes were calculated on the assumption that the specific activity of the ion throughout the plant and solution system remained constant. Knowledge of this value, and the rate of loss or gain of activity of the bathing solutions, determined by the slope of the graph, were the only parameters needed for the simple calculation (Appendix 3).

The value for influx was obtained by addition of the efflux value, to that of the net flux, as net influx = influx - efflux.

The results are tabulated in Table 7.

Table 7.

Bathing solution	ionic concentration	Net influx	Efflux	Influx	Flux ratio (in/out)
$\frac{1}{16}$ sea water	65 mEq Cl/l	5.95×10^{-7}	6.02×10^{-8}	6.55×10^{-7}	10.88
½ "	325 "	1.94×10^{-6}	2.81×10^{-7}	2.22×10^{-6}	7.90
Full sea water	650 "	1.44×10^{-5}	1.04×10^{-6}	1.54×10^{-5}	14.80
$\frac{1}{10}$ sea water	52 mEq Na/l	4.91×10^{-7}	3.05×10^{-8}	3.54×10^{-7}	11.27
½ "	260 "	4.60×10^{-6}	4.85×10^{-7}	5.08×10^{-6}	10.43
Full	520 "	1.37×10^{-5}	2.24×10^{-6}	1.59×10^{-5}	7.08

Flux measurements are all expressed as mEq/second

The pattern of the flux ratios obtained are as predicted from the data obtained in Section 4 (a), and it would be expected that the indications of active accumulation of Cl, and export of Na would also be correct. However, as it has been shown that flux equilibrium was not likely to have been attained in the previous experiments, it is of value to contrast the actual fluxes obtained with those predicted by the Ussing-Teorell equation, if ion transport is passive. The Ussing-Teorell equation can be stated thus:-

$$\frac{Jin}{Jout} = \frac{Co}{Ci}\ exp.\ \left(\frac{ZFE}{RT}\right)$$

where Jin, Jout = influx and efflux respectively

$\frac{Jin}{Jout}$ = flux ratio

Co = ionic concentration in the medium

Ci = " " " W.F.S.

Z = valency

F = 96.5 coulombs/equivalent

T = temperature, Kelvin

R = 8.31 joules/degree/equivalent

The predicted flux ratio ($\frac{in}{out}$) are compared with those actually obtained in Table 8.

Table 8.

Na			Cl		
Bathing medium	Predicted	Actual	Bathing medium	Predicted	Actual
1/10	106.9	11.27	1/10	2.516×10^{-5}	10.88
½	521.1	10.43	½	3.152×10^{-4}	7.90
1	1078	7.08	1	7.057×10^{-5}	14.8

Conclusions

Thus it would appear that Na is actively excreted from the plant roots, as the values for the flux ratio obtained are less than those predicted if Na was passively distributed. Similarly, that Cl is actively pumped in, as the values obtained are enormously in excess of those predicted. The rate of uptake of ^{36}Cl increased, as predicted, at full sea water concentration, when compared with the rate at ½ sea water concentration.

It would appear that conclusions reached in (a) have been substantiated by the results obtained using radio-active tracers.

Figure 29 A

Log. Na concn. in W.F.S. vs. external concn.

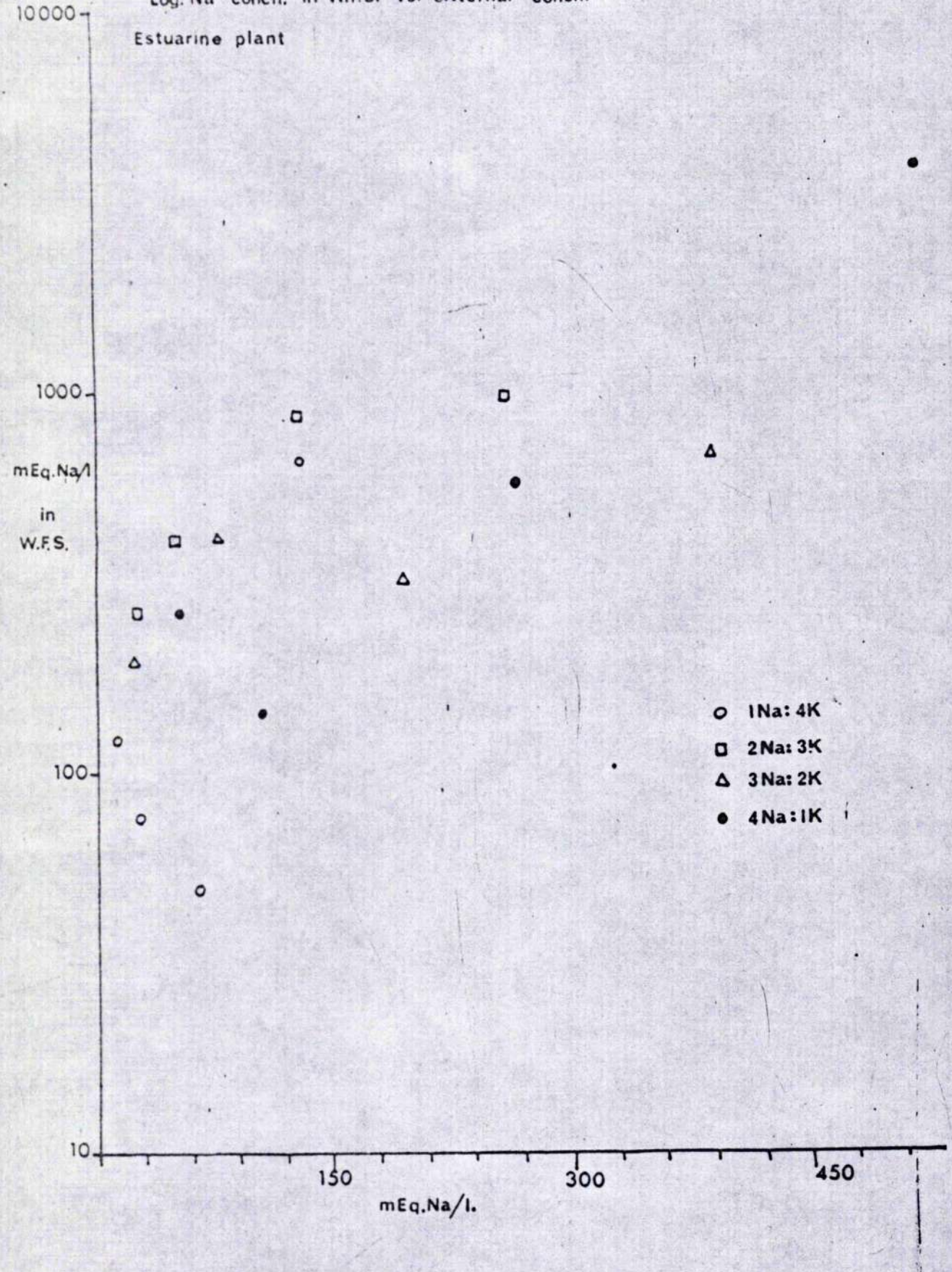


Figure 29 B

Log. Na concn. in W.F.S. vs. external concn.

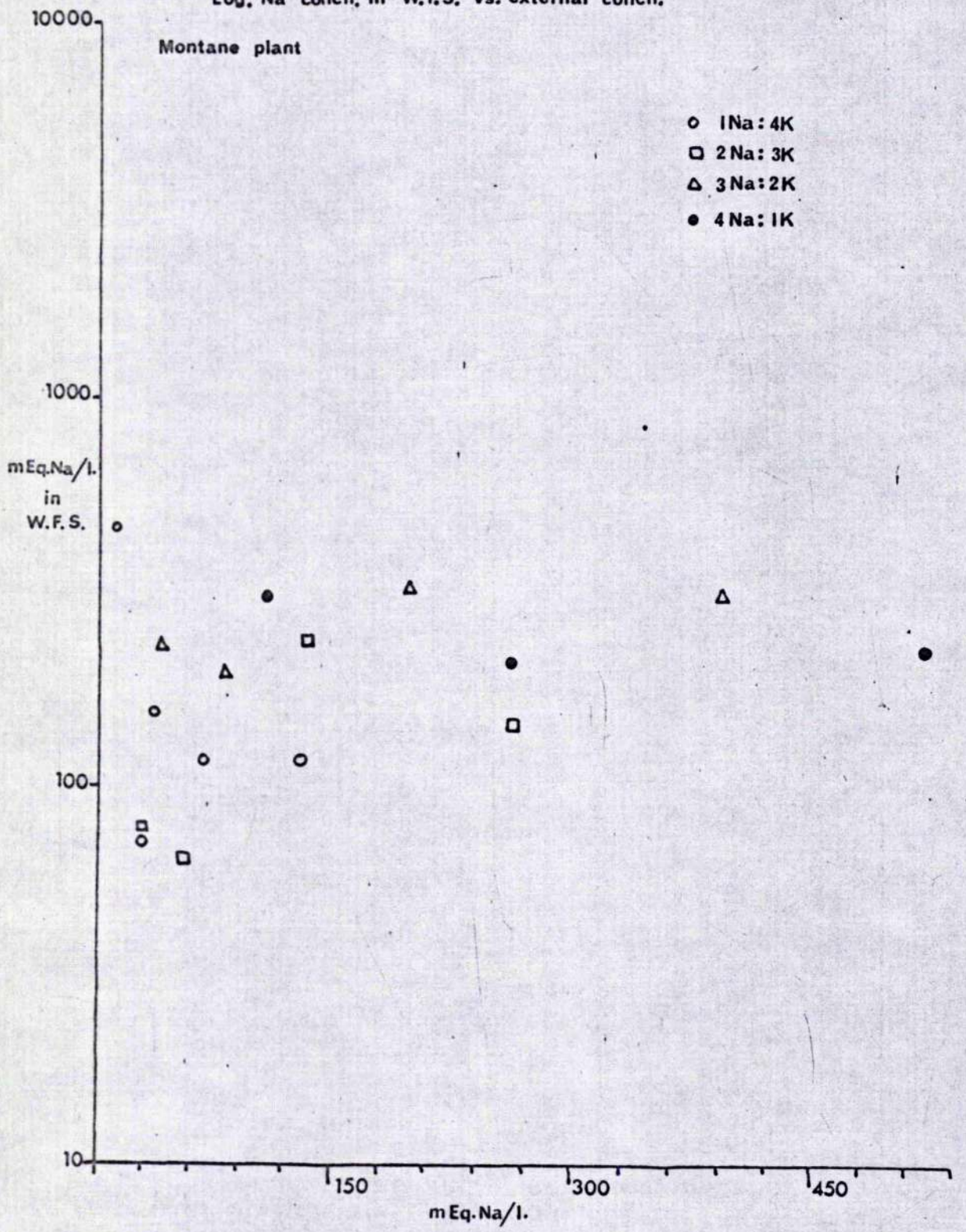


Figure 29

Log. K concn. in W.F.S. vs. external concn.

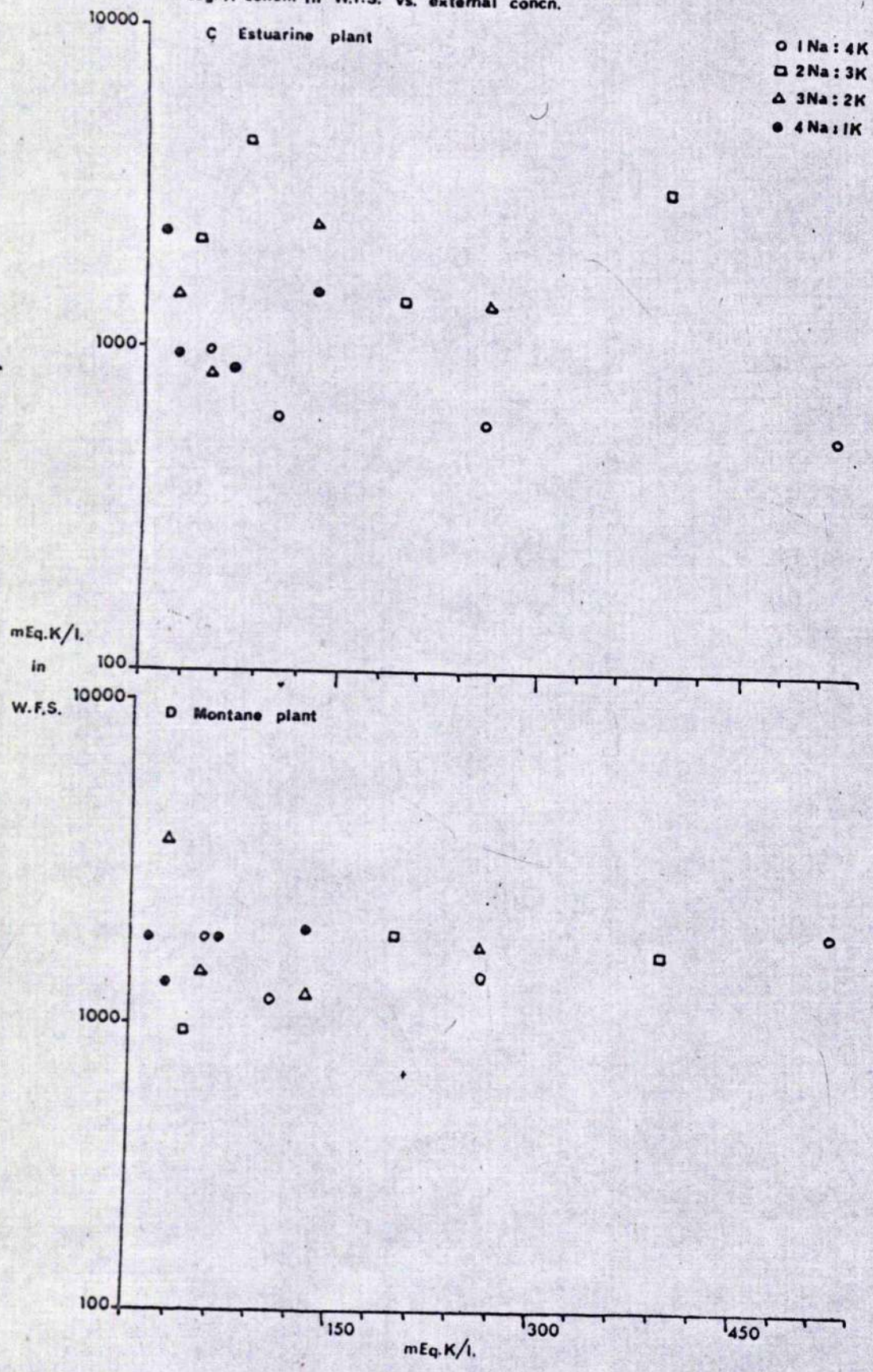
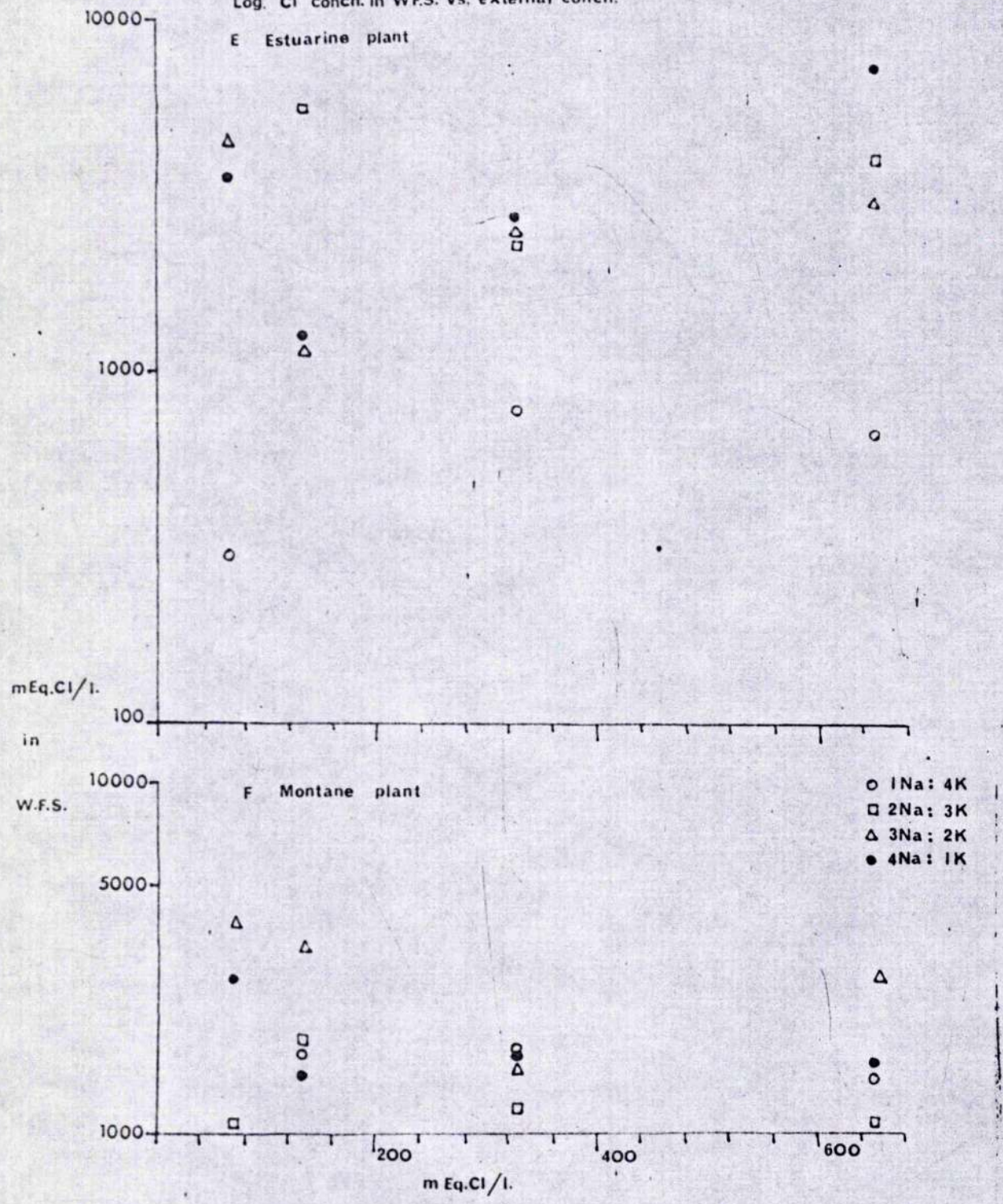


Figure 29

Log. Cl concn. in W.F.S. vs. external concn.



c) Values of E obs - E calc. related to changes in concentration of ions in the W.F.S., with increase in concentration of the bathing media.

If the ion concentration in the W.F.S. was not controlled at either the root or the leaf, it would be expected that the concentration of each ion in the W.F.S., would increase with increase in external concentration. The existence of 1 or more regulatory mechanisms should be apparent on study of this relationship.

Figures 29 A - F represent the concentration of 1 ion in the W.F.S., at the concentration of this ion in the bathing medium. The symbols used are definitive of the cation ratio in the external medium, as explained for Figures 28. It can be seen that a steady concentration of Na is registered in the leaf W.F.S. of montane plants at high external concentrations. Similarly, the level of K and Cl in the leaves of plants from both ecotypes, is maintained at a constant level. This level is of a noticeably higher concentration than for Na. The concentration of Na in the W.F.S. of estuarine leaves was not nearly so constant.

The tendency of these Figures to show a constant level of ions in the sap, as the concentration of the solution bathing the roots increased, is a function of the mechanism, or mechanisms, regulating ion uptake at the root, or excreting ions from the leaves. Values calculated for E obs. - E calc. for each ion in (a), can be

related to the concentration in the W.F.S., but do not define the site of regulation.

If the change in concentration of Na in the W.F.S. of leaves is considered, it can be seen that in a montane plant, initial values for E obs. - E calc. were in the region of the zero line. That is, the ion was in approximate electrochemical equilibrium. Thus, as the external concentration increased slightly, the concentration in the W.F.S. also increased. However, at higher concentrations, the value for E obs. - E calc., became more negative, and the concentration level in the W.F.S. became stable. Thus active export of Na, causing the fall in E obs - E calc., resulted in a plateau value being attained for concentration in the W.F.S.

This situation can be contrasted with that in the estuarine leaf. Initially, the behaviour in this plant was similar to that in the montane. Increase in external concentration of Na up to 200 mEq/l. showed some indication of a steady concentration being maintained in the W.F.S., although the level was greater than in the montane. Some values for E obs. - E calc. up to this concentration did show more negative values, related to Na excretion by the plant. However, further increase in external concentration caused the internal concentration of the xylem sap to increase, with a corresponding movement of E obs - E calc. towards zero.

This behaviour can be ascribed to a high membrane permeability to Na, so that at high concentrations, inward diffusion was too rapid for compensation by the outwardly directed ion pumps. That is, the ion pumps were swamped.

Active import of K has been shown to occur at low external concentrations, and to show a decline in value towards passive distribution, and perhaps active excretion, at a higher concentration of K in the bathing medium. However, the value for K concentration in the W.F.S. is very constant with increase in external concentration, especially in the montane leaf. (Figure 29D).

It can be postulated that active accumulation at low external concentration, was effective in raising the concentration in the leaf W.F.S. to the level shown in the graph. However, as the concentration of K in the bathing solution increased, it is possible that the membrane permeability to K also increased, causing an increase in the loss of K, through leakage from the cell. This leakage increased progressively with increase in the concentration in the bathing medium, so that the tendency to accumulate ions decreased, maintaining a steady K concentration in the W.F.S. A similar mechanism will explain the constancy of the internal Cl level.

However, active excretion of K has been shown to be a possible interpretation of Figures 28 C and D at high external

concentrations. Although the above mechanism is a simple explanation of the maintenance of constant internal K concentration, demanding only 1 inwardly directed K pump, a more complicated model may have to be invoked. By the second model a constant K concentration in the W.F.S. would be explained by the operation of a mechanism for active accumulation at low concentrations, followed by passive movement, and active excretion as the concentration in the medium increased.

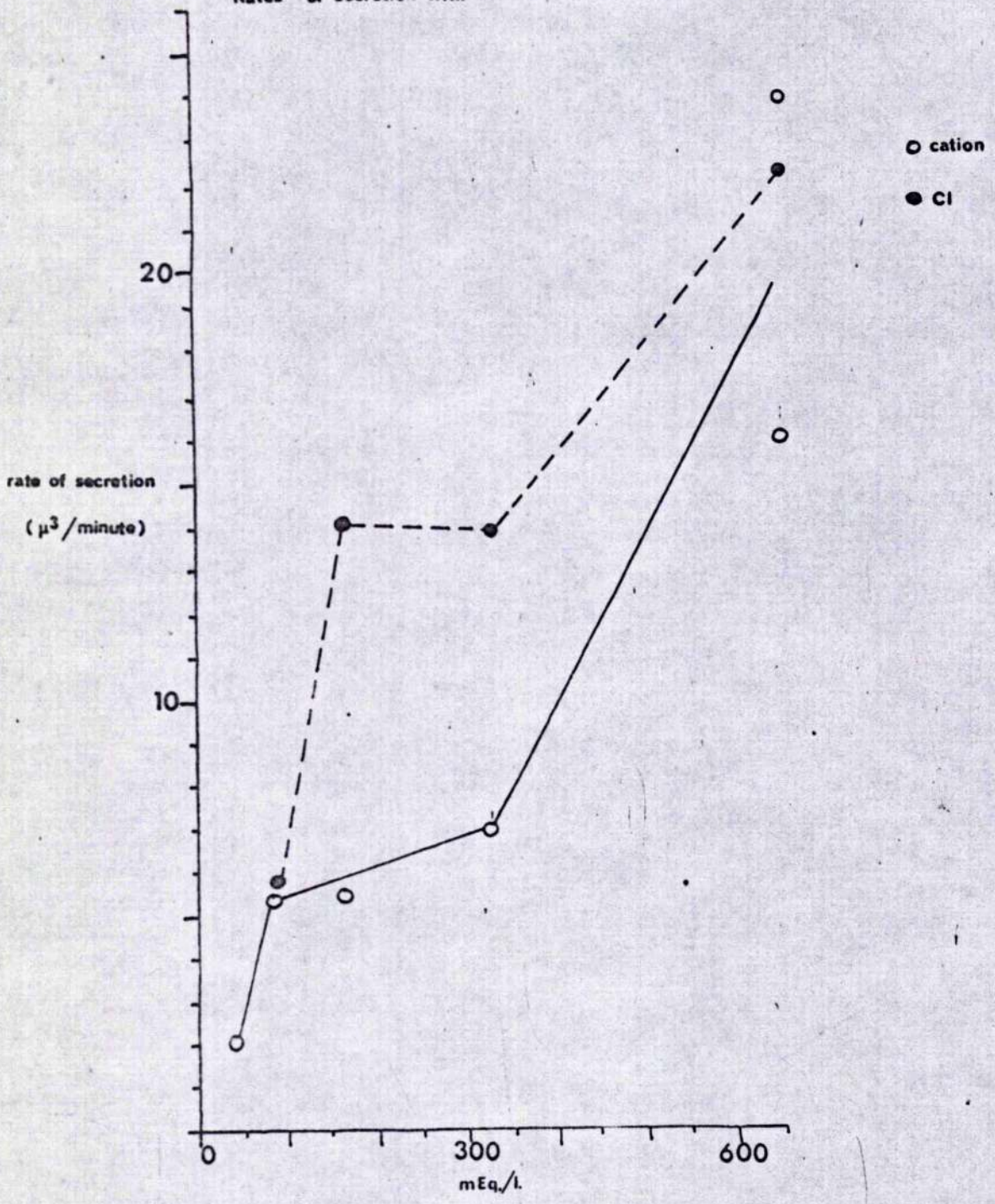
The location of regulatory mechanism, or mechanisms, has not been identified by this study. The possibility of regulation by the root, or by the leaf glands, or both, still remains.

d) The gland as an organ of excretion

White crystals were observed on the surfaces of leaves above the glands, as the concentration of the bathing medium was increased to the levels of 1/5, ½, and total artificial sea water. Crystals were removed from the surface of leaves of experimental plants by brief washing with distilled water. Leaves from which crystals were analysed, were adjacent to those from which concentrations of ions in the W.F.S. were measured. The values for ion ratios in the crystals were 1 Na:4.01K and 1Na:6.33 Cl, when the cationic composition of the bathing solution was 1Na:4K, and 1Na:0.093K and 1Na:1.10Cl

Figure 30

Rates of secretion with increase in ion concentration



when the medium was 4Na:1K. However, the number of crystals was greater with higher Na content in the bathing medium. Relative values of Na and K excreted for equivalent external concentrations were 1:0.47.

Thus it would appear that the glands were capable of excretion of K, and that at identical concentrations in the bathing medium, approximately twice as much Na was excreted as was K.

This data does not provide evidence of the efficiency of the glands as de-salinators.

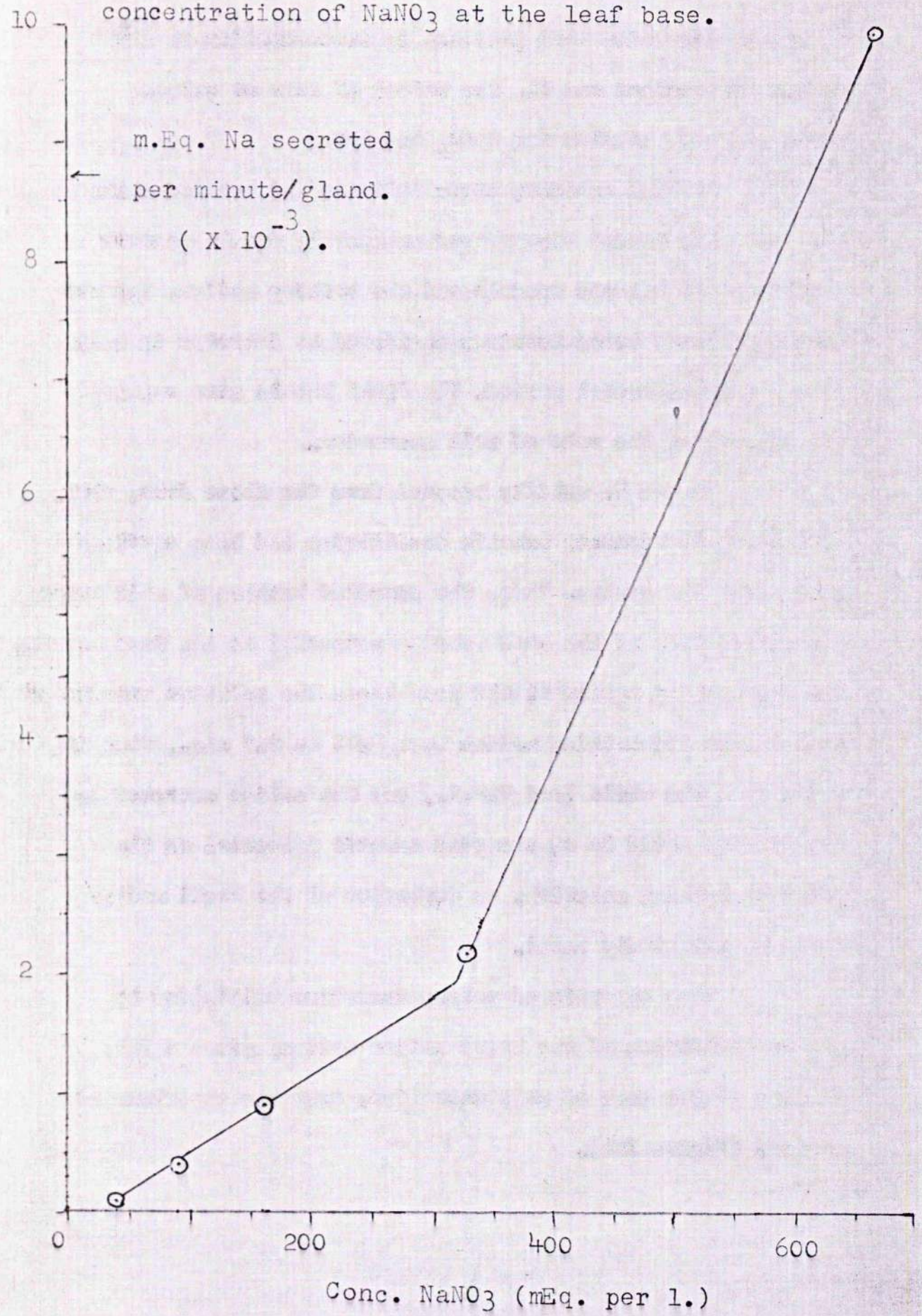
a) Rates of gland excretion

The apparatus has been described in Chapter 2. Section 8. An initial attempt to use the method outlined by Hill (1967) failed, because lens tissue was found to contain elutable Na and K, in varying amounts.

Leaves from montane plants were used, and solutions of $NaNO_3$, KNO_3 and choline chloride over a concentration range of 30-630 mEq/l., applied to the leaf bases. The diameter of the bubbles of secretion, which were assumed to be spherical because the density difference between water and paraffin was low, were measured after a period of 60-90 minutes. The volume of the bubble, and hence the rate of volume secretion, assuming this to be constant, could be calculated. Figure 30 shows values for the rate

Figure 30 A

Rate of secretion of Na with increase in concentration of $NaNO_3$ at the leaf base.



of volume secretion with increase in concentration of the medium for cations and Cl. The values of rate of volume secretion were similar for $NaNO_3$ and KNO_3.

At high concentrations (650 mEq/l.) bubbles which had initially formed disappeared presumably due to osmotic equilibration between exudate and the bathing medium. Exudate formed at lower concentrations continued to increase in size over the experimental period. The final bubble size was used for estimating the rate of salt secretion.

It can be validly assumed from the above data, that, during the 90 minutes, osmotic equilibrium had been attained throughout the system. Thus, the excreted bubbles of salt above the glands were of the same osmotic potential as the leaf W.F.S., and the bathing medium at the leaf base. The relative volumes of leaf tissue and bathing medium were 0.02 to 0.5 mls., that is, 1:25. Thus the whole leaf W.F.S., and the saline excreted by the glands, would be at the same osmotic potential as the original bathing solution, as depletion of the basal medium would be relatively small.

Thus the rate of volume excretion multiplied by the concentration of the basal saline medium, gives a fair measure of the rate of salt secretion, over the experimental period. (Figure 30A).

Figures 30 and 30 A appear to confirm Hill's (1967) results with Limonium vulgare, that all 3 major ions can be excreted. The high rates of secretion corresponding with high external concentration, were based on bubble size 10 minutes after the start of the experiment, and hence before osmotic equilibrium had occurred. For this reason, these results are not considered to be an accurate indication of gland efficiency at high concentrations.

Whereas Hill obtained hyperbolae for rate of ion secretion with increase in the concentration of the basal medium, these results for Armeria maritima would seem to indicate a linear relationship for similar concentration ranges.

f) Efficiency of the glands as de-salinators

The fact that the glands on the leaf surface can excrete salt, by pumping each of the major ions, has been established. What has not been established is whether the glands played a significant part in the maintenance of a stable internal ionic concentration in the leaf W.F.S.

This efficiency was tested in the following manner. Crystals were removed from 3 leaves, which were adjacent to those from which elution from the W.F.S. was performed. From the resulting analysis the total salt content of the W.F.S., and crystals, of an "average" plant were calculated. These

values were then compared with the total salt content of the ambient media. An "average" plant was defined as having 14 "heads", each bearing 30 leaves.

The data is tabulated below (table 9), for estuarine and montane plants in concentrated saline containing 4Na:1K, and for the estuarine plant in concentrated saline of composition 2Na:3K. Thus a plant (montane) which maintained a steady internal salt concentration, was contrasted with one (estuarine), which did not, over a concentration range which stimulated glandular excretion. The third plant was chosen as an example of an estuarine plant, with a steady concentration of salt in the W.F.S., at the higher concentrations of bathing media used.

Table 9.

		TOTAL SALT CONTENT				
Plant and Medium	Medium (m Moles)	relative increase	W.F.S. (m Moles)	relative increase	CRYSTALS (m Moles)	relati incre
Estuarine	78	1.0	55.2	1.0	0.014	1.0
4Na:1K	195	2.5	96.6	1.75	0.244	17.3
	360	4.6	165.2	3.0	0.868	62.0
Montane	78	1.0	43.7	1.0	0.008	1
4Na:1K	195	2.5	48.8	1.11	0.014	1.75
	360	4.6	50.4	1.17	0.008	1
Estuarine	78	1.0	5.18	1	0.014	1
2Na:3K	195	2.5	105	20.27	0.504	36
	360	4.6	106	20.46	0.746	53.2

If these results are examined for individual plants, it can be seen that, for the estuarine plant in 4Na:1K medium, the relative increase in salt content of the W.F.S., increased more slowly than that in the external medium. This can be contrasted with the relative increase in salt excreted, which was greater than the relative increase in salt in the W.F.S. However, the absolute amount excreted is very small in comparison with the amount of increase in the W.F.S.

Similarly, for a montane plant in the same solution, the relative values for salt content in both tissue and crystal, remain approximately constant with increase in external salt content. The absolute amount of salt excreted was negligible, in comparison with the absolute increase in content of the bathing medium.

The estuarine plant growing in 2Na:3K medium was chosen, as an example of a plant which exhibited a steady internal salt content, at high external concentrations. Thus, an initially high relative increase in salt content in the W.F.S., resulted in a stable level being maintained on further increase in external concentration. Again, the salt content of the crystals increased by greater relative proportions, but in absolute terms contributed little in comparison with the increase in the salt content of the bathing medium.

In this comparative study of 3 plants, each behaving

differently with increase in ionic content of the bathing medium, it was obvious that increase in salt content of the W.F.S., did stimulate excretion from the glands. However, the part played by the glands in maintaining a steady internal concentration was shown to be negligible, in terms of actual amount of salt excreted.

g) Discussion

It had originally been planned to measure the electrochemical potential for each ion in the xylem sap, collected by exudation from a cut stem, or root. Experimental difficulties, which have been described in Chapter 2, Section 3, precluded this approach. Subsequently, ion concentrations in the leaf W.F.S. were measured, so that values of the electrochemical potential calculated for each ion, over a range of external concentrations, might not be a direct indication of the effect of the root as a permeability barrier. Crystals, which have been observed to form on the leaf surface, must be the result of an excretion mechanism. Therefore, the electrochemical potentials for each ion in the W.F.S., were indicative of selective processes taking place in the root, and in the leaf.

Results obtained in this Section appear to indicate, however, that the part played by the leaf in ion regulation in the plant, is minor. It would appear, therefore, that the

main site of active regulation occurs in the root, and that values obtained for the electrochemical potential of ions in the W.F.S., can be attributed, in the main, to regulation by the root.

Each of the major ions has been shown to be capable of stimulating gland secretion at similar concentrations, when applied to the base of a detached leaf(e). These results are in accord with those of Hill (1967), working with another member of the Plumbaginaceae, Limonium vulgare. However, it would seem that increase in the concentration of Na, in the W.F.S. of an attached estuarine leaf, promoted excretion in Armeria maritima. This excretion occurred, even though the concentration of the Na in the leaf W.F.S., was lower than that of K or Cl.

In order to explain, it must be postulated that either; in vivo the glands are stimulated by lower concentrations of Na, than of the other ions, or, increase in Na caused an increase in the concentration of K or Cl in the W.F.S., which in turn stimulated secretion.

Concurrently with the experimental determinations of E obs. - E calc., a series of analyses were conducted of the relative ion contents of the W.F.S., cytoplasm, vacuoles, and crystals, of other leaves on the plant 'head' which was under experimental observation. These results are tabulated in Appendix 4.

It was clear from these results that the ratio of K/Cl in the vacuoles of leaf cells remained constant, in the region of 1, irrespective of the K/Na ratio in the medium. Thus an increase in Na in the W.F.S. could cause an increase in Cl concentration in the W.F.S., as NaCl could not be accumulated by leaf vacuoles, without upsetting the constancy of K/Cl.

Therefore, gland excretion may be stimulated by an indirect mechanism, caused by increase in the Cl. concentration in the W.F.S., which was a direct consequence of increase in Na concentration. Values for Cl concentration in the W.F.S. of estuarine leaves were not so constant as those for montane leaves, so values obtained in Figure 29E may not be definite.

However, an increase in net influx of ^{36}Cl by estuarine plants, was observed on increase in the external concentration from ½, to total artificial sea water.

Lack of electrochemical data reduces the hypothesis presented above to the speculative level, but it would appear to be consistent with the facts obtained. Arisz et al. (1955), have claimed to show, again without electrochemical evidence, that Cl is actively excreted from the glands of Limonium latifolium.

General Discussion

Chapter 4.

The results have been discussed in Chapter 3, in the context of the separate sections. The work of these sections will now be discussed in more general terms, in relation to the regulation by the plants, of their salt content.

In this discussion, absorption of ions from xylem sap by the living cells of the plant, other than of the young cortex, had been deliberately omitted. Whilst these cells undoubtedly absorb ions from the xylem sap, and are shunt reservoirs for ions, they will not affect the ionic contents of xylary sap, in any steady state condition of dynamic flux equilibrium throughout the plant.

a) Electrochemical potentials of ions in the leaf W.F.S., and the effects of increase in concentration of the bathing medium.

It has been shown in this study, that Na is actively excreted at all but possibly the lowest external concentrations, and that Cl is actively accumulated at all external concentrations. The electrochemical gradient against which Cl is moved, decreased as concentration in the bathing medium increased. K is actively accumulated at low external concentrations, is in approximate

passive electrochemical equilibrium, at higher concentrations, and is possibly excreted actively at very high external concentrations.

These results for Armeria maritima are similar to those obtained using an electrochemical approach, by Shone (1969), Bowling, Macklon and Spanswick (1966), and Etherton (1961), working on Zea, Ricinus, and Avena spp. The concentration ranges used in this work on Armeria were, however, much greater than those used in work on other species.

b) Sites of regulation of ion content in the plant

Three tissues have been proposed as being sites of ion regulation. Two of these occur in the root, and were detected by purely electrical measurements, the third being situated at the leaf glands. Comparative measurements of ion content have suggested, that the glands cannot function at a sufficient rate to enable them to control the ionic content of the whole plant. Thus it would seem that the ionic content of the plant is controlled by 1, or more permeability barriers, situated in the root.

The exodermis and endodermis

In dilute culture media, an electrical potential was observed between the intercellular spaces of the cortex, and the bathing medium. A similar potential has been reported by Ginsberg and Ginsberg (1967), in Zea mays.

Repeated penetration of an electrode through the exodermis did not cause this electrical potential to be reduced, as may have been expected; the reduction being caused by short circuiting through a perforation to the bathing medium. Upon increase in the concentration of the bathing medium, this potential disappeared. Thus the electrical potential recorded from the intercellular spaces had all the characteristics of a simple Donnan potential. However, it was noticed that the potential did re-appear with time, after increase in external concentrations of the medium, up to that of ½ sea water.

This later evidence suggested that the potential across the exodermis may not be solely passive in origin, but that the cells of the exodermis and cortex may exert some active control over it.

In contrast, the potential across the endodermis did disappear on repeated penetration, and did not reduce to a great extent on increase in concentration of the bathing medium. It was concluded that the endodermis did regulate actively, but the relative efficiency of the endodermis and exodermis as barriers to free ion movement was not known.

Permeability studies

Values of the permeability of the exodermis to the major ions were obtained, over range of 1-100 mEq/l. Loss of

turgor by the protoplasts, probably caused by the prolonged root growth in a very dilute medium, the concentration of which was suddenly increased, precluded accurate measurement of membrane permeability at higher concentrations. However, further experimentation showed that change in exodermal potential, with change in the cation ratio of artificial sea water, was of the order of that predicted from values of permeability at lower concentrations (Chapter 3, Sections 2 and 3).

It was reasoned that if the permeability of the exodermis was a main barrier to free movement of ions into the root, then the relative permeability of the exodermis to the major ions, would be comparable with that shown by the whole root.

Inclusions of these permeability values into the Goldman equation, predicting the value for the electrical potential between leaf and solution, would, if the value calculated approximated to that actually measured, be consistent with the exodermis being the main barrier to ion flow.

The Goldman equation is stated thus:

$$E = RT \ln. \frac{pNa(Nao) + pK(Ko) + pCl(Cli)}{pNa(Nai) + pK(Ki) + pCl(Clo)}$$

where pNa, pK, pCl refer to the permeabilities of the exodermal membrane to Na, K, Cl, respectively. The brackets and suffixes,

o, and i, refer to the concentration of the ions in the external medium, and leaf W.F.S., respectively.

Values of E calculated from the Goldman equation, and those actually obtained are tabulated below, (table 10).

Table 10

ESTUARINE			MONTANE	
Concn.	E.obs.	E (Goldman)	E.obs	E (Goldman)
1/10	-54	-31	-63	-11
1/5	-45	-33	-70	-18
½	-40	-17	-66	-21
1	-54	-26	-57	-18

It would appear that a discrepancy exists between values for E predicted and obtained. In all cases the predicted value was less than that actually recorded.

To change the value of E calculated from the Goldman equation, to that observed, pNa and/or pK must be greater, or pCl less, than the value calculated. Since pNa and pCl are much smaller than pK (1,0.35, 2.15 respectively, for the estuarine exodermis), and Cl and Na are far from electrochemical equilibrium, and presumed to be actively transported; alterations in the passive permeabilities of these ions would have less

effect than changes in pK, with increase in distance into the root; K being near to passive electrochemical distribution. It can be concluded that for any given values of pNa and pCl, the inner tissues are relatively more permeable to K than the exodermis.

Hence, the exodermis may not be the sole barrier to ion movements between a root and the xylary sap.

Tracer studies

If cellular-controlled fluxes can control the concentration of an ion in the neighbourhood of a cell, in either the cortex or the exodermis, such an explanation being necessary to explain the recovery of intercellular potentials; then it would be predicted that passive initial influx should not be much greater than net flux into the xylem. That is, a first phase of passive movement into the root would occur, which would be followed by a slower net flux; efflux having begun.

The bi-phasic uptake observed in Chapter 3, Section 4 (b) cannot be ascribed to such a situation. The initial flux was of too great a duration and the difference in flux ratio for the 2 phases, was too great to fulfil the above model. It must also be remembered that a 2 hour delay occurred in each experiment, before meaningful results were

obtained, because of adsorption and stirring artefacts.

It would appear therefore, that initial uptake observed at the above concentrations was into a distinct spatial phase, and was most likely to have been uptake into the cortex.

If it is assumed that in this experimental state the exodermis is not a barrier to free diffusion, then the intercellular spaces outside the endodermis will be A.F.S. in the true sense of the definition, and will contain bathing medium at the same concentration and activity, as outside the root.

If it is assumed also, that the concentration of ion in the cytoplasm and vacuole of the cortical cells is the same (no difference in electrical potential exists at these concentrations - Chapter 3 Section 3), then the approximate concentration of the labelled ion in each cortical cell can be calculated.

Comparison of Cl and Na fluxes into the cortex with those into the xylem.

It was estimated for sections, that 15/24 of the volume of the whole root was occupied by exodermal and cortical cells. The volume of root immersed in bathing medium is known, as is the total uptake into phase 1, and the specific activity of the bathing medium. Thus the approximate concentration of ion

in the cortical and exodermal protoplasts can be calculated.

Data obtained from Chapter 3, Section 3, can be utilised to obtain values for the electrical potential of the cortical cells in bathing media of the same composition, as those containing the tracers. Hence, this data can be applied to the Ussing-Teorell equation to predict values for flux ratio for an ion which is passively distributed. Comparison with the actual flux ratios, calculated from the net flux into phase 1, assuming efflux to be constant for both phases of uptake, will yield information on both the direction and magnitude of active constraint on the ion.

These values can be compared with those obtained for ion movement into the xylem (table 11).

Table 11

ion and solution	Predicted flux ratio into cortex	Actual flux ratio into cortex	Predicted ratios into xylem	Actual ratios xylem
^{36}Cl 1/10	7.73×10^{-2}	29.56	2.516×10^{-5}	10.88
^{36}Cl ½	3.73×10^{-2}	20.59	3.152×10^{-4}	7.90
^{22}Na 1/10	117.4	45.27	106.9	11.27

The deviation of the actual from the predicted flux ratios is indicative of the efficiency of the ion regulatory mechanisms exist at the endodermis, and the combined cortex and exodermis.

It can be seen that the actual flux into the xylem of ^{22}Na is approximately 1/1o that predicted, whereas into the cortex the flux is over 1/3 of the value predicted. Similarly, for values with ^{36}Cl, the actual intake into the xylem compared with that predicted, is some 2 orders of magnitude greater, than for the similar occurrence in the cortical cells.

Thus, although tissue outside the endodermis regulates ion uptake in directions which are identical with endodermis, the degree of regulation is much less outside the endodermis. It can be concluded therefore, that as a barrier to free diffusion of ions into a plant, the endodermis is much more efficient than the exodermis.

c) Significance of a barrier to free diffusion of ions at the exodermis

Free diffusion of ions is undoubtedly limited at the exodermis in dilute media. The existence of this barrier at high concentrations of the bathing medium was not tested after a sufficient time had elapsed for equilibrium within the root. It must be remembered that the plant roots had been bathed by a dilute medium for at least 18 months prior to sudden increase in concentration. This was part of the experimental design, whereby one of the objectives had been a comparison of the response of plants, from both montane and estuarine habitats, to a saline and osmotic shock.

Thus, the initial response to an osmotic shock, is one of protoplast plasmolysis. This in turn results in an increase in the size of the pathway to free inward movement of ions, caused by withdrawal of the protoplast from the closely fitting radial cell walls of the endodermis. So, at the moment when the free diffusion pathway into the plant should be as small as possible, it is at its widest.

There is no way of knowing from the data which has been obtained, whether control of ion uptake, if present as an active mechanism at the endodermis, was re-established during the course of the experiment when the roots were bathed in sea water, or whether it is maintained when the protoplasts are of sufficient osmotic potential to withstand osmotic shock, as in the normal estuarine environment.

It may, however, be postulated that the existence of free water in the intercellular spaces, will be of ecological significance in buffering the protoplasts of the cortex against dehydration in the estuarine environment. Such dehydration may be expected to occur if sudden evaporation of the soil solution occurs, causing this to become concentrated, so having a higher osmotic potential than that of the plant protoplasts.

d) The exodermis in relation to interpretation of uptake by the root in other studies

Most other studies on root uptake (Lattes, 1959, Hylmö 1955, 1958, Lundegårdh 1950, Shone 1969), have been made on plants growing in media which are dilute in comparison with the concentration range used in this work. Ginsberg and Ginsberg (1967) have shown that a barrier to free diffusion of ions exists at the exodermis of Zea mays. It may therefore, be reasonable to assume that such a barrier will exist at the exodermis of most plants grown in relatively dilute media, even though this barrier to free diffusion is likely to be the result of a Donnan system.

Thus it would seem that Hylmö's (1955) assertions of individual root cells acting individually as "unicellular submerged algae", may be disputed. The results seem to confirm Lundegårdh's (1953) view of the exodermis as a permeability barrier. Without reliable electrochemical data his conclusions of active transport at this barrier, cannot be tested, however.

Shone (1969) has stated that values he has obtained for ion fluxes into the stele, are often not proportional to the electrochemical driving forces on the ion concerned in sap exudate. These driving forces were measured between exudate

collected in a capillary (House and Findlay, 1966), and the bathing medium; the exudate being taken to represent xylem sap, the endodermis being the site of the ion regulation mechanism.

However, if the solution in the intercellular spaces has a higher osmotic potential than the bathing medium, it is quite possible that exudation may occur from the cortical spaces, as the exudate was collected a bare 2 mms. above the level of the bathing solution. Thus the solution collected would be a mixture of ions which had, and had not crossed the endodermis, and the electrical potential recorded would be a combination of that across the combined exodermis and endodermis, and that across the exodermis alone. Thus the electrochemical potentials he records, will be a complex function of cellular interactions across the root, as he himself suggests, although not for the same reasons.

It might appear, therefore, that for such experiments to lead to valid conclusions on ion transport to the stele, a method must be found to seal the cortex from the collecting pipette.

e) <u>The glands</u>

It has been shown that the glands are not capable of dealing with a large influx of ions to the leaf (Chapter 3, Section 4 (f)). However, the activity of the glands is increased with increase in salt concentration in the leaf W.F.S. It may be

assumed, therefore, that the glands act as a mechanism for controlling slight increases in leaf salinity, possibly due to salt spray penetrating the stomata as an aerosol. Thus the significance of the salt glands from an evolutionary viewpoint, is debateable. However, the possibility does exist that the efficiency of the glands in field-grown plants, is greater than in laboratory-grown specimens.

No evidence was found of regulation of salt content by abscission of leaves containing high concentrations of salt. Leaf abscission always occurred in January to February, irrespective of the salt status of the plants.

f) Conclusions

It appears that Armeria maritima regulates its ionic content in a manner intermediate between the extremes described by Atkinson et. al. (1967), for the mangroves, Rhizophora mucronata and Aegialitis annulata. The former has no salt glands and excludes salt from the xylem at the roots, the latter allowing some salt into the xylem, and regulating this by excretion from the leaves.

Armeria maritima grown in the laboratory over a period of 3 years, could not regulate its ion content solely by excretion from the leaves. However, the 2 or more permeability barriers in the root; situated at the endodermis, exodermis, and possibly the sum of the living cortical cells; were capable of maintaining an internal ionic concentration, which could be tolerated by the plant. In some laboratory-grown specimens, the roots of montane

plants appeared to be more efficient in controlling salt uptake, than those of estuarine plants.

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Appendix 1.

Values of concentration of ions in the bathing medium and in the leaf W.F.S.

	External concentration (mEq/l).			Conc in W.F.S. Estuarine (mEq/l).			Conc in W.F.S Montane (mEq/		
Basic solution	Na	K	Cl.	Na	K	Cl	Na	K	
1Na:4K	13	52	65	121	969	303	363	1818	
	26	104	130	76	613	-	70	1197	1
	65	261	325	38	593	760	114	1379	1
	130	521	650	648	521	650	118	1952	1
2Na:3K	26	39	65	267	2142	-	71	935	1
	52	78	130	305	4444	3555	63	1189	1
	130	196	325	851	1390	2305	236	1908	1
	260	391	650	957	3052	3947	140	1619	1
3Na:2K	39	26	65	196	1464	4464	229	3750	4
	78	52	130	413	827	1127	192	1438	3
	195	131	325	316	2455	2531	322	1250	1
	390	261	650	685	1385	2857	301	1792	2
4Na:1K	52	13	65	262	2261	3571	152	1819	2
	104	26	130	140	955	1123	310	1310	1
	260	66	325	571	863	2678	206	1855	1
	521	131	650	3888	2555	6666	326	1894	1

Appendix 2.
(not quoted in text)

Ratios of ion concentration in the W.F.S. to ion concentrations in the bathing medium, over the complete range of external concentrations

	Na external conc. (mEq/l)	Na in/out ES.	Na in/out MON.	K external conc. (mEq/l)	K in/out ES.	K in/out MON.	Cl external conc. (mEq/l)	Cl in/out ES.	Cl in/out MON.
S2	13	9.307	27.923	52	18.634	34.961	65	4.661	-
	26	2.923	2.692	104	5.894	11.509	130	-	13.538
	65	0.584	1.753	260.7	2.27	5.28	325	2.338	5.304
	130	4.984	0.907	521.4	0.999	3.743	650.2	0.999	2.275
S3	26	10.269	2.730	39	54.923	23.974	65	-	16.476
	52	5.865	1.211	78	56.974	15.243	130	42.736	14.6
	130	6.546	1.815	195.7	7.102	9.74	325	7.092	3.523
	260	3.680	0.538	391.4	7.797	4.136	650.2	6.070	1.624
S4	39	5.025	5.871	26	56.307	144.230	65	68.676	64.092
	78	5.294	2.461	52	15.903	27.653	130	8.669	26.984
	195	1.620	1.651	130.7	18.783	9.563	325	7.787	4.806
	390	1.756	0.771	261.4	5.298	6.855	650.2	4.394	4.352
S5	52	5.038	2.923	13	173.923	139.923	65	54.938	42.723
	104	1.346	2.980	26	36.73	50.384	130	8.638	11.538
	260	2.196	0.792	65.7	13.135	28.234	325	8.24	4.756
	520	7.476	0.626	131.4	19.444	14.414	650.2	10.252	2.426

Appendix 3.

Sample calculation of net influx of ^{22}Na from ½ sea water

Influx = 5.69 cpm/hour.

Activity = 954 cpm/2.6 mEq. Na.

$$\text{uptake /second} = \frac{5.69 \times 2.6}{954 \times 3600}$$

$$= \underline{5.08 \times 10^{-6} \text{ mEq Na/second.}}$$

APPENDIX 4

EXTERNAL CONCN.	EXTERNAL			A. ESTUARINE PLANTS												B. MONTANE PLANTS											
				W.F.S.			CYTOPLASM			VACUOLE			CRYSTALS			W.F.S.			CYTOPLASM			VACUOLE			CRYSTALS		
	K/Na	Cl/Na	Cl/K	K/Na	Cl/Na	Cl/K	K/Na	Cl/Na	Cl/K	K/Na	Cl/Na	Cl/K	K/Na	Cl/Na	Cl/K	K/Na	Cl/Na	Cl/K	K/Na	Cl/Na	Cl/K	K/Na	Cl/Na	Cl/K	K/Na	Cl/Na	Cl/K
1/10	4·01	5	1·25	16	—	—	19	4·37	2·30	12	15	1·25	—	—	—	31·2	30	0·96	11·2	15	1·33	33·77	17·77	0·52	—	—	—
	1·51	2·5	1·66	5·76	8	1·38	4·4	32·5	7·38	4·34	5·17	1·19	—	—	—	6·55	10	1·52	8·8	—	—	38·5	22·5	0·58	—	—	—
	0·69	1·69	2·49	6·9	21·4	3·1	5	18·18	3·63	12·58	12·90	1·02	—	—	—	11·90	18·18	1·52	6·2	—	—	44·83	43·54	0·97	—	—	—
	0·25	1·25	4·95	6·4	15	0·42	1·62	4	2·46	11·7	10	0·85	—	—	—	5·06	9·37	1·85	4	—	—	11·96	10·29	0·86	—	—	—
1/5	4·01	5	1·25	8·73	—	—	6·17	—	—	15·45	10·9	0·7	8·8	50	5·68	14·4	15	1·04	8·8	—	—	28·88	14·58	0·50	—	—	—
	1·51	2·5	1·66	11·91	4·54	0·38	5	—	—	13·87	14·51	1·04	1·81	13·63	7·5	19	30	1·57	4	30	7·5	37·14	18·57	0·50	—	—	—
	0·69	1·69	2·49	4·7	7·5	1·59	4·6	7·5	1·63	8·66	10	1·15	0·8	7·5	9·37	13·8	30	2·17	1·6	15	9·37	15·12	13·12	0·86	—	—	—
	0·25	1·25	4·95	6·9	20	2·89	8·8	30	3·4	18	20	1·11	0·72	13·63	18·75	5·87	12·50	2·12	4	15	3·75	4·4	7·5	1·70	1·6	15	9·37
1/2	4·01	5	1·25	5·24	8	1·52	5·09	11·36	2·23	1·68	2·25	1·19	2·3	6·7	2·69	17	15	0·88	6·2	15	2·41	19·21	13·15	0·68	1·33	—	—
	1·51	2·5	1·66	2·94	0·98	0·34	1·76	—	—	1·65	2·30	1·38	0·94	1·80	1·91	6·25	5	0·74	4·96	3	0·62	6·22	6·22	1	0·81	0·63	0·77
	0·69	1·69	2·49	19·4	25	1·29	2·66	1·66	0·62	1·62	2·96	1·82	0·48	3·04	1·47	8·1	7·5	0·92	6·2	15	2·41	12·76	9·80	0·76	2·24	3	1·33
	0·25	1·25	4·95	1·7	5·25	3·08	2·02	6·25	3·08	1·19	2·40	2·02	0·17	3	16·93	3·25	4·65	2·06	4·4	—	—	8·16	5·93	0·68	16	3·75	0·23
1	4·01	5	1·25	4·62	5·55	1·20	5·11	4·44	0·86	5·27	7·27	1·37	4·01	6·33	1·57	18	16·25	0·90	17·63	18·18	1·03	11·56	9·56	0·85	8·43	10·93	1·29
	1·51	2·5	1·66	2·20	2·66	1·21	3·48	3·03	0·86	1·13	2·08	1·84	0·72	1·63	2·24	10·66	12·90	1·28	11·2	30	2·67	4·39	6·69	1·52	4	18·18	4·54
	0·69	1·69	2·49	1·92	3·46	1·80	2·19	4·28	1·95	1·63	2·80	1·71	1·21	3·28	2·70	8·18	9·37	1·14	5·06	20·31	4·01	7·85	6·70	0·85	0·97	1·66	1·70
	0·25	1·25	4·95	1·94	1·66	3·24	0·56	1·76	3·12	0·16	0·57	3·44	0·93	1·10	11·8	5·38	6·94	1·28	4·68	9·37	2	3·39	2·65	0·78	3·02	19·44	6·44